ANNUAL REPORT OF PROGRAM ACTIVITIES NATIONAL CANCER INSTITUTE

Fiscal Year 1975

Volume III

(Part III B)

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

Fiscal Year 1975

Volume III

(Part III B)

U.S. NATIONAL CANCER INSTITUTE

Report of program of activities.

Part III B - Division of Cancer Cause and Prevention

KC
267
U562d
v.3
pt 3B
1975

SUMMARY REPORT

Part III-B

OFFICE OF THE ASSOCIATE DIRECTOR FOR VIRAL ONCOLOGY (OADVO)

July 1, 1974 - June 30, 1975

Dr. J. B. Moloney

The Annual Report for Viral Oncology, Division of Cancer Cause and Prevention, NCI, is presented as follows:

A. Viral Oncology Area - The Virus Cancer Program (VCP)

1. Introduction
2. Organization
   a. Viral Oncology Area
   b. Virus Cancer Program

3. Adminstrative Highlights
4. Scientific Activities
   a. Narrative
   b. Progress Highlights

5. Projections
6. Meeting Reports
7. Bibliography

B. Summary Reports

1. Frederick Cancer Research Center
2. Offices of the Associate Director for Viral Oncology
   a. Office of Biohazards and Environmental Control
   b. Office of the Coordinator for Ultrastructural Studies
   c. Office of Program Resources and Logistics
   d. Scientific Information Management Unit

3. Branch Reports

   a. Viral Biology Branch
      (1) Summary
      (2) Individual Project Reports

   b. Viral Carcinogenesis Branch
      (1) Summary
      (2) Individual Project Reports

   c. Viral Leukemia and Lymphoma Branch
      (1) Summary
      (2) Individual Project Reports

C. The Virus Cancer Program Contract Summaries by Segments

1. Breast Cancer Virus
   a. Summary
   b. Individual Contract Reports

2. Developmental Research
   a. Summary
   b. Individual Contract Reports

3. Immuno-Epidemiology
   a. Summary
   b. Individual Contract Reports

4. Solid Tumor Virus
   a. Summary
   b. Individual Contract Reports

5. Tumor Virus Detection
   a. Summary
   b. Individual Contract Reports

6. Program Resources and Logistics Reports

7. Program Management Reports

A. Viral Oncology Area - The Virus Cancer Program (VCP)

1. Introduction

The Viral Oncology Area is responsible for planning and conducting the Institute's program of coordinated research on viruses as etiological agents of cancer. Scientists within this Area not only provide the broad operational management for intramural and collaborative research but also conduct investigations on oncogenic viruses and their interaction with host cells. Any information obtained from these coordinated studies is rapidly applied to (1) search for viruses or virus genetic information which may be etiologically related to the initiation of human cancer and (2) develop therapeutic and preventive measures for control of human cancers when such causative agents are found. This program, as it is now structured, contributes to six of the seven goal-oriented and key objectives set forth in the National Cancer Plan, the ultimate goal of which is control of all human cancers.

Contract-supported research is conducted within the Viral Oncology Program under the Virus Cancer Program (VCP). The yearly budget from the inception of this Program in 1964 to the present and the number of professional positions are given below.

Funding History - VCP Contracts
(in thousands)

Fiscal Year	Positions	VO	SVLP	VCP	Totals
64	30	4,926	--	--	4,926
65	90	5,433	8,723	--	14,156
66	95	3,064	13,556	--	16,620
67	138	3,137	13,505	--	16,642
68	140	--	--	17,241	17,241
69	157	--	--	17,985	17,985
70	167 (59)*	--	--	17,340	17,340
71	187 (68)	--	--	31,591	31,591
72	226 (79)	--	--	41,889	41,889
73	217 (83)	--	--	42,564	42,564
74	221 (84)	--	--	49,553	49,553
75	220 (85)	--	--	49,885	49,885

* Figures in parentheses represent professional staff, GS-13 and above.

The Program has succeeded in marshaling many of the nation's finest virologists, biochemists, immunologists, molecular biologists, epidemiologists, and physicians for this strongly goal-oriented effort. From the beginning, it was clear that an understanding of the suspected relationship between tumor viruses and human neoplasia would not only require an interaction among these groups of scientists but sound and constructive administrative support as well. For a complete history of this Program, see previous Annual Reports.

The Research Logic for the VCP, based on the premise that a virus or viral genetic information persists in the host and is an indispensable element for the induction of certain kinds of human cancer, is continually updated. The plan is reviewed regularly by the Director, NCI; the Director, Division of Cancer Cause and Prevention, NCI; the National Cancer Advisory Board, NCI; the Executive Committee, NCI; and the Cause and Prevention Executive Staff, NCI. In FY 74, at the request of the Viral Oncology Area, the National Cancer Advisory Board appointed an ad hoc committee to review the research efforts of the VCP and suggest any relevant changes. Last year, following acceptance of the ad hoc committee's report, the Chairman, NCAB, appointed a subcommittee composed of three Board members to assist in the implementation of this review.

2. Organization

a. Viral Oncology Area

From its inception, the Viral Oncology Area and the Virus Cancer Program were organized into an integrated structure which manages both intramural and collaborative research studies. All efforts have been coordinated by the Office of the Associate Director with its three Offices and three Branches, each with several sections (and units) and program segments. Each segment was assisted by a working group with members outside the federal government.

Current Intramural Organization (Fiscal Year 1975)

Office of the Associate Director

Science Management Team

Dr. J.B. Moloney, Associate Director for Viral Oncology
Dr. L.R. Sibal, Deputy Associate Director, VO
Dr. Elke Jordan, Coordinator for Collaborative Research, VO
Dr. H.J. Hearn, Scientific Coordinator for Viral Oncology, Frederick Cancer Research Center

Administrative Staff

Mr. John Miller, Administrative Officer, DCCP
Mr. N.A. Olimpio, Program Analyst, DCCP
Mr. Robert Velthuis, Administrative Officer, VO
Mrs. Linda Christoferson, Administrative Assistant, VO

Office of Biohazards and Environmental Control

Biohazards Research Section
Environmental Control Section

Office of Coordinator for Ultrastructural Studies

Viral Studies Section

Office of Program Resources and Logistics

Viral Biology Branch

Cell Biology Section
Microbiology Section
Experimental Pathology Section
Human Tumor Studies Section
Virus and Disease Modification Section

Viral Leukemia and Lymphoma Branch

Primate Virus Section
Tumor Virus Section
Chemical Studies Section
Viral Pathology Section
Genetics Section
Viral Biochemistry Section

Viral Carcinogenesis Branch

Molecular Biology Section
Ecology and Epizoology Section
Solid Tumor Virus Section
Viral Genetics Section

With the concurrence of the Director, NCI, and the Director, DCCP, and under the guidance of the Office of Management Analysis, OD, NCI, the Viral Oncology Area is planning a major reorganization of its intramural structure. These changes are being effected with existing personnel, space and funds, and are needed to: (1) provide new opportunities for inhouse scientists who deserve and want more responsibility; (2) attract new scientists and program leaders; (3) take cognizance of the changes in scientific emphasis and opportunities and maintain high productivity among scientific personnel; and (4) effect a separation in the operations of the intramural (VO) and the collaborative (contract - VCP) research programs. Since the entire plan has not yet been implemented, only the major organizational framework is provided below.

Proposed Intramural Organization (Fiscal Year 1976)

Office of the Associate Director

Office of Biohazard Safety
Office of Program Resources and Logistics

Laboratory of Tumor Virus Genetics

Laboratory of RNA Tumor Viruses

Laboratory of DNA Tumor Viruses

Laboratory of Viral Carcinogenesis

Collaborative Research Branch

b. The Virus Cancer Program

Background. The responsibility for the overall management of the Virus Cancer Program is within the Office of the Associate Director, Viral Oncology; the Associate Director is the Chairman, Virus Cancer Program. To maintain effective management of individual or groups of collaborative studies, this Office has been assisted by the Chairmen and Vice-Chairmen of five Program Segments: (1) Breast Cancer Virus; (2) Developmental Research; (3) Immuno-Epidemiology; (4) Solid Tumor Virus; and (5) Tumor Virus Detection. Collectively, these Chairmen constituted the Program Segment Chairmen, a body responsible for planning, implementing and reviewing contracts for relevance, priority and need within the total Program. The Segment Chairmen were drawn from senior intramural scientists who were asked to serve the Virus Cancer Program as scientific managers or advisers and to maintain active intramural research programs. Peer group review of contract proposals for scientific merit and technical excellence was conducted by Working Groups composed of a majority of non-government scientists.

Present Operations. During FY 1975 the Chairman, Virus Cancer Program (VCP) took two major steps which have changed the review practices significantly. These are (1) the chartering of two VCP Scientific Review Committees (A and B) and (2) the chartering of a VCP Advisory Committee.

The first two Committees, A and B, consist of up to 25 members each, including the Acting Chairman. The members, all non-NCI scientists, were appointed by the Director, NCI to serve overlapping terms of four years on the basis of their outstanding qualifications and recognized expertise in the fields of microbiology, molecular biology, immunology, biochemistry and virology, as related to cancer. The purpose of these Committees is to review individual proposals for scientific excellence and technical competence. NCI staff are no longer voting members of these Committees and participate only as needed in the review process. The Committees operate entirely under the guidelines set forth by the OMB, DHEW, NIH and NCI regulations.

The VCP Advisory Committee, consisting of seven members including the Chairman, will be appointed by the Director, NCI to serve overlapping terms of four years. Again the members are chosen entirely from the biomedical community on the basis of their qualifications and expertise in the cancer field, in particular cancer virology. The mission of the Committee is to advise the Chairman, VCP, on broad directions for the Program under the National Cancer Plan for the Conquest of Cancer. Particular emphasis is placed on allocation of resources, areas for expanded research and development, and the application of research findings to the control of cancer in man. The Committee's efforts are separate from the review and approval of individual contracts.

Review Committees A and B have met regularly during the past year. The rosters are given below:

Virus Cancer Program Scientific Review Committee A

To review contracts in Developmental Research, Breast Cancer Virus, and Immuno-Epidemiology Segments.

Dr. Marcel Baluda, University of California (Los Angeles)
Dr. David Bishop, Rutgers University (New Brunswick, NJ)
Dr. Arthur Brown, University of Tennessee (Knoxville)
Dr. Andrew G. Dean, Arkansas Department of Health (Little Rock)
Dr. Bernice Eddy, Retired, Bureau of Biologics, NIH
Dr. Donald Fine, Frederick Cancer Research Center
Dr. Paul Gerber, Bureau of Biologics, NIH
Dr. Raymond Gilden, Flow Laboratories (Rockville, MD)
Dr. Eva Klein, Karolinska Institute (Sweden)
Dr. David Kohne, Scripps Clinic & Research Foundation (La Jolla, CA)
* Dr. Mathilde Krim, Sloan-Kettering Institute for Cancer Research (NYC)
Dr. Arleen Levin, Rush-Presbyterian-St. Luke's Medical Center (Chicago)
Dr. Miriam Lieberman, Stanford University School of Medicine (Palo Alto)
Dr. Frank Lilly, Albert Einstein College of Medicine (NY)
Dr. Hans Meier, The Jackson Laboratory (Bar Harbor, ME)
Dr. William C. Moloney, Peter Bent Brigham Hospital (Boston)
Dr. Richard H. Morrow, Jr., Harvard School of Public Health (Boston)
Dr. Malcolm Pike, USC School of Medicine (Los Angeles)
Dr. William E. Rawls, McMaster University Medical School (Canada)
Dr. Kirby L. Smith, St. Jude Children's Research Hospital (Memphis)
Dr. Kenneth K. Takemoto, NIAID, NIH
Dr. Lise Thiry, Institut Pasteur du Brabant (Belgium)

Virus Cancer Program Scientific Review Committee B

To review contracts in Solid Tumor Virus and Tumor Virus Detection Segments.

Dr. J. Thomas August, Albert Einstein College of Medicine (NY)
Dr. Paul H. Black, Massachusetts General Hospital (Boston)
Dr. Dani Bolognesi, Duke University Medical Center (Durham, NC)
Dr. Janet S. Butel, Baylor University College of Medicine (Houston)
Dr. Robert Chanock, NIAID, NIH
Dr. Joseph D. Feldman, Scripps Clinic & Research Foundation (La Jolla)
** Dr. Charlotte Friend, Mt. Sinai School of Medicine (NY)
Dr. Clarence J. Gibbs, Jr., NINDS, NIH
Dr. Adeline J. Hackett, University of California (Berkeley)
Dr. Francoise Haguenau, College de France (France)
Dr. Janet Hartley, NIAID, NIH
Dr. Gertrude Henle, Childrens Hospital of Philadelphia
Dr. Jacob Holper, Litton-Bionetics (Kensington, MD)
Dr. Edmund Klein, Roswell Park Memorial Institute (Buffalo)
Dr. Henry Kunkel, The Rockefeller University (NYC)
Dr. Henry T. Lynch, Creighton University School of Medicine (Omaha)
Dr. Paul E. Neiman, University of Washington (Seattle)
Dr. Marvin A. Rich, Michigan Cancer Foundation (Detroit)

---

* Acting Chairman, Committee A ** Acting Chairman, Committee B

The VCP Advisory Committee is expected to hold its first meeting in the near future. While the members have not yet been appointed officially, the preliminary roster is given below:

Virus Cancer Program Advisory Committee (to be appointed)

Dr. Emil Frei III, Children's Cancer Research Foundation (Boston)
Dr. Charlotte Friend, Mt. Sinai School of Medicine (NYC)
Dr. Daniel Nathans, Johns Hopkins School of Medicine (Baltimore)
Dr. Lloyd Old, Sloan-Kettering Institute for Cancer Research (NYC)
* Dr. Frank Putnam, University of Indiana (Bloomington)
Dr. William S. Robinson, Stanford University Medical Center (Palo Alto)
Dr. Robert Wagner, University of Virginia Medical School (Charlottesville)

* Chairman

Intramural Review Committees (Fiscal Year 1975)

Program Segment Chairmen

Dr. J.B. Moloney
Dr. James Duff
Dr. Maurice Guss
Dr. Alfred Hellman
Dr. Robert Huebner
Dr. Elke Jordan
Dr. Paul Levine
Dr. Robert Manaker
Dr. Wade Parks
Dr. Gary Pearson
Dr. Jeffrey Schlom
Dr. Edward Scolnick
Dr. Louis Sibal
Dr. Bernard Talbot
Dr. George Todaro

Program Resources and Logistics Advisory Group

Dr. Jack Gruber, Chairman
Dr. David Howell, Executive Secretary
Dr. Robert Bassin
Dr. James Duff
Dr. Peter Fischinger
Dr. Maurice Guss
Dr. Robert Holdenried
Dr. Garrett Keefer
Dr. Gary Kelloff
Dr. Wade Parks
Dr. Gary Pearson
Dr. George Vande Woude

Program Management Segment

Dr. J.B. Moloney, Chairman
Dr. L.R. Sibal, Executive Secretary
Dr. Michael Chirigos
Dr. A.J. Dalton
Dr. James Duff
Dr. Jack Gruber
Dr. Alfred Hellman
Dr. Robert Huebner
Dr. Robert Manaker
Dr. Bernard Talbot
Dr. George Todaro

Frederick Cancer Research Center Advisory Group

Dr. Henry J. Hearn, Chairman
Dr. James Duff
Dr. Maurice Guss
Dr. Jack Gruber
Dr. Louis Sibal
Dr. Bernard Talbot
Mr. Robert Velthuis

Contract Specialists (Members of Research Contracts Branch, NCI)

Mr. William Caulfield
Mr. Charles Fafard
Mr. John Gibbons
Mr. Sidney Jones
Mr. Jacques Labovitz
Mr. J. Thomas Lewin
Mr. Thomas Porter
Mr. Clyde Williams

Consultants

Dr. Mumtaz Ahmed, Pfizer, Inc. (Maywood, NJ)
Dr. Ralph B. Arlinghaus, M.D. Anderson Hospital & Tumor Inst. (Houston)
Dr. Roland Aronson, Johns Hopkins University (Baltimore)
Dr. Thomas August, Albert Einstein College of Medicine (NY)
Dr. Laure Aurelian, Johns Hopkins University (Baltimore)
Dr. Marcel Baluda, UCLA Cancer Center (Los Angeles)
Dr. William Baxt, University of California (San Diego)
Dr. John Berg, University of Iowa (Iowa City)
Dr. David Bishop, Rutgers University (New Brunswick, NJ)
Dr. Francis Black, Yale University School of Medicine (New Haven)
Dr. Paul Black, Massachusetts General Hospital (Boston)
Dr. Michael J. Brennan, Michigan Cancer Foundation (Detroit)
Dr. Dani Bolognesi, Duke University (Durham, NC)
Dr. Arthur Brown, University of Tennessee (Knoxville)
Dr. Arsend Burny, Faculte des Sciences Agronomique de l'Etat (Belgium)
Dr. Janet Butel, Baylor University College of Medicine (Houston)
Dr. Robert Cardiff, University of California (Davis)
Dr. David Colcher, Meloy Laboratories (Springfield, VA)
Dr. Mark Chatighny, Naval Biological Laboratory (Oakland, CA)
Dr. Ronald Dorfman, Stanford University Medical Center
Dr. Bernice Eddy (Retired, NIH), Bethesda, MD
Dr. Myron E. Essex, Harvard University School of Public Health (Boston)
Dr. Joseph D. Feldman, Scripps Clinic & Research Foundation (La Jolla)
Dr. William Feller, Georgetown University Medical Center (Washington, DC)
Dr. Donald Fine, Frederick Cancer Research Center
Dr. Bernhard Fleckenstein, Universitat Erlanger/Nurenberg (West Germany)
Dr. Charlotte Friend, Mt. Sinai School of Medicine (NYC)
Dr. Benjamin R. Forsyth, University of Vermont (Burlington)
Dr. Anthony Girardi, Wistar Institute (Philadelphia)
Dr. Raymond Gilden, Flow Laboratories (Rockville, MD)
Dr. Alan Garen, Yale University (New Haven)
Dr. Donald Gustafson, Purdue University School of Medicine (Lafayette, IN)
Dr. Adeline Hackett, University of California (Oakland)
Dr. Francoise Haguenau, College de France (France)
Dr. Yoshiyuki Hashimoto, Tokyo Biochemistry Research Institute (Japan)

Dr. William Hardy, Sloan-Kettering Institute for Cancer Research (NYC)
Dr. Gertrude Henle, Children's Hospital of Philadelphia
Dr. Jacob Holper, Litton-Bionetics (Kensington, MD)
Dr. Ronald Hunt, New England Regional Primate Center (Southborough, MA)
Dr. Hidesaburo Hanafusa, The Rockefeller University (NYC)
Dr. James Ihle, Frederick Cancer Research Center
Dr. Al Jonas, Yale University School of Medicine (New Haven)
Dr. Raymond Kahn, University of Michigan (Ann Arbor)
Dr. Edmund Klein, Roswell Park Memorial Institute (Buffalo, NY)
Dr. Eva Klein, Karolinska Institutet (Stockholm)
Dr. David Kohne, Scripps Clinic & Research Foundation (La Jolla)
Dr. Mathilde Krim, Sloan-Kettering Institute for Cancer Research (NYC)
Prof. Gerhard R.F. Kruegar, University of Cologne (Germany)
Dr. Donald Kufe, Institute for Cancer Research (NYC)
Dr. Henry Kunkel, The Rockefeller University (NYC)
Dr. Miriam Lieberman, Stanford University Medical Center (Palo Alto)
Dr. Frank Lilly, Albert Einstein College of Medicine (NY)
Dr. Arleen Levin, Rush-Presbyterian-St. Luke's Medical Center (Chicago)
Dr. Henry Lynch, Creighton University School of Medicine (Omaha)
Dr. Robert M. McAllister, Children's Hospital of Los Angeles
Dr. Edward Melby, Johns Hopkins University School of Medicine (Baltimore)
Dr. Joseph L. Melnick, Baylor University College of Medicine (Houston)
Prof. George S. Michaelson, University of Minnesota (Minneapolis)
Dr. William Moloney, Peter Bent Brigham Hospital (Boston)
Dr. Richard Morrow, Harvard School of Public Health (Boston)
Dr. Carel Muldes, Cold Spring Harbor Laboratories (NY)
Dr. Hans Meier, The Jackson Laboratory (Bar Harbor, ME)
Dr. D.B. Nayak, University of California (Los Angeles)
Dr. Andre Nahmias, Emory University (Atlanta)
Dr. Paul Neiman, Providence Hospital (Seattle)
Dr. Malcolm Pike, USC School of Medicine (Los Angeles)
Dr. Harvey Rabin, Litton-Bionetics (Kensington, MD)
Dr. Fred Rapp, Milton S. Hershey Medical Center (Hershey, PA)
Dr. William Rawls, McMaster University Medical Center (Canada)
Dr. Marvin Rich, Michigan Cancer Foundation (Detroit)
Dr. Harry M. Rose (Retired), Center Harbor, NH
Dr. Albert Sabin, Medical University of South Carolina (Charleston)
Dr. Joseph Sambrook, Cold Spring Harbor Laboratories (NY)
Dr. George Santos, Johns Hopkins University (Baltimore)
Dr. Jerald Schochetman, Meloy Laboratories (Springfield, VA)
Dr. Michael Shimkin, University of California (San Diego)
Dr. Kirby Smith, St. Jude Children's Research Hospital (Memphis)
Dr. Calude Tihon, Frederick Cancer Research Center
Dr. Lise Thiry, Institute Pasteur du Brabant (Belgium)
Dr. George G. Wright, Massachusetts Dept. Public Health (Jamaica Plains)
Dr. Ernest Wynder, American Health Foundation (NYC)
Dr. David Yohn, Ohio State University (Columbus)

Administrative Structure of VCP

Segment Chairmen and Vice Chairmen. The segment chairmen are responsible for planning the projects in each working group. As senior scientists, they must review, analyze and integrate studies which fulfill the objectives of their working group and the total Program.

Executive Secretaries. Executive Secretaries assist the chairmen in managerial duties of contract operation. They are responsible for optimal review and complete documentation of each project within the working group.

Project Officers. Project Officers are the direct extension of program segment chairmen. To assure progress in accomplishing the goals set forth in the workscope of a project, they are called upon to advise principal investigators on scientific matters and coordinate segment and program decisions.

Review Committees (Working Groups). The program segment working groups are the peer review units for the Program. The committees review individual contracts or proposals for scientific excellence and technical competence within a given funding level. Their recommendations provide the Segment Chairmen, Associate Director for Viral Oncology, and the Director, DCCP, with a basis for program decisions.

Contract Specialists. Contract specialists are responsible for negotiating research contracts. They provide valuable advice on fiscal and legal matters to the project officers, executive secretaries, segment chairmen, and Associate Director. Some specialists are well conversant with the scientific aspects of the Program. They are assigned to Program Areas by the Research Contracts Branch, NCI.

Contract Review. The Projects within the total Program are reviewed at many levels:

1. Each contract is reviewed for relevance, priority, and need to total Program by the Program Segment Chairmen.

2. Each contract is reviewed for scientific excellence and technical competence by the Program Segment Working Group.

3. Each contract is continually monitored for performance by the Project Officer.

4. Each contract above the annual funding level of $1 million and with multifaceted workscope undergoes a third review by an ad hoc committee appointed by the Associate Director for Viral Oncology.

5. Each contract is reviewed by the Associate Director for Viral Oncology; the Director, Division of Cancer Cause and Prevention; the Chief, Research Contracts Branch, NCI; and, as required, by the Director, NCI.

As an aid to the review processes, key staff members receive progress reports on all contracts on a biannual basis. Collection and distribution of these reports is the responsibility of the Information Unit in the Office of the Associate Director. A single comprehensive report is prepared annually by the Associate Director.

Future Operations. It is anticipated that the current committee structure will remain after reorganization of intramural personnel has taken place. However, the establishment of a new Collaborative Research Branch (see Proposed Intramural Organization) will produce yet another significant change in the handling of contracts within the Virus Cancer Program. While the overall responsibility for Program direction will remain within the Office of the Associate Director for Viral Oncology, collaborative studies will be administered primarily by the Chief, CRB, and his staff. Contracts currently in established Segments will be redistributed among newly created sections according to the general scope of the work on the contracts. For the first time, the VCP will use a new funding mechanism, the Cancer Research Emphasis Grant (CREG). By definition, the studies supported by this mechanism must fit into the framework of the VCP objectives. Although the studies will be goal-oriented (targeted), the means of reaching the goal will be investigator-designed, thus requiring less direction and management on the part of the Program staff than contracts. A more complete explanation of CREG can be found in several documents emanating from the Office of the Director, NCI.

3. Administrative Highlights

The FY 75 budget was accompanied by more uncertainties than usual. The NCI appropriation of $691 million was made in December, already late in the fiscal year. The President immediately requested from the Congress the recission of a large portion of that appropriation to an NCI level of $568 million. The Congress voted not to honor the President's request and the full amount of the appropriation was released. Because of this additional delay in the appropriation cycle, a firm operating budget was not obtained until the beginning of the fourth quarter. This has made financial planning extremely difficult. The result for Viral Oncology has been a budget level approximately the same as the previous year. If the recission request had been approved, the Viral Oncology Program would have experienced a reduction of approximately $2.4 million. Even with a substantial increase in the NCI appropriation from $600 million in FY 74 to $691 million in FY 75, the Viral Oncology Program received no significant increase because of a shift in program emphasis within the Division.

No new personnel positions accompanied the increase in budget. In fact, NCI's operating personnel ceiling was reduced by NIH. The effect on Viral Oncology was the loss of three positions. This was one factor contributing to a substantial overstrength for the year. The other factor was the hiring of several additional employees to occupy six locations in which space had been leased to support Viral Oncology investigators serving as on-site project officers at local contract sites.

The Viral Carcinogenesis Branch's Poolesville Trailer Unit was relocated to FCRC. The Biohazards Research Section of the Office of Biohazards and Environmental Control was relocated from the Open Bay Area of Building 41 to FCRC. A contract was negotiated and work was begun in the late Spring on the construction of permanent containment laboratories within the Open Bay Area of Building 41. The space should be ready for occupancy in approximately two years, and will be used to return some investigators to NIH who have previously conducted their research in rented space at local contract sites while serving as on-site project officers at these locations.

4. Scientific Activities

A. Narrative

Some of the major developments in the field of tumor virology during the past year are included in this section of the report. Emphasis is placed on contributions made by intramural and collaborative (contract) scientists.

RNA Viruses

Oncogenic RNA viruses belong to a class of RNA viruses distinguished by having an RNA-directed DNA polymerase (RDDP). At present, two major groups are recognized: (1) type C viruses and (2) type B viruses. The two groups, differentiated by their morphology and the diseases they cause, appear to show no crossreaction by immunologic and nucleic acid hybridization techniques. Viruses of both groups (1) mature by budding through the cell membrane, (2) have a density of 1.15-1.19 in sucrose gradients, (3) have 70S RNA as their genome, and (4) contain RDDP. Besides the two major groups, a few viruses have been identified that seem to fall somewhere between type C and type B viruses in their morphologic characteristics, e.g., Mason-Pfizer Monkey Virus (MP-MV) and guinea pig endogenous virus.

Type C Viruses. Type C viruses generally fall into two classes--leukemia and sarcoma viruses. Both occur in a wide variety of species in which they cause leukemias, lymphomas and related neoplasms. Although all leukemia viruses can infect, replicate and mature independently, most mammalian sarcoma viruses are defective. The latter can transform cells which they infect but cannot replicate except in the presence of a helper leukemia virus. The leukemia virus supplies the functions lacking in the sarcoma virus but does not itself have the ability to transform cells. Many leukemia and sarcoma stocks are probably mixtures or recombinants of several viruses. Recent experiments have demonstrated that the various type C viruses recombine readily and that RNA from one type can be packaged in a coat of another type. It appears plausible that a transforming virus could be formed when a non-oncogenic virus picks up "transforming information" either from its host or from virus integrated into the host genome.

Type B Viruses. The most extensively studied type B virus is the Mouse Mammary Tumor Virus (MMTV) which causes mammary adenocarcinoma in mice. Characterization of this virus has lagged behind that of type C viruses because of the lack, until recently, of a system for large-scale growth of the virus in cell culture. Since it has recently become possible to grow MMTV to high titer, great strides have been made in studies of the virus. A hormone, dexamethasone, has a strong stimulatory effect on viral replication. The hormone appears to increase the rate of transcription as measured by viral RNA synthesis. This is the only known case where a hormone has been shown to stimulate preferentially transcription of a specific RNA.

Exogenous and Endogenous Viruses. Both type C and type B viruses can be either exogenous or endogenous in origin. Exogenous viruses are transmitted horizontally or by artificial infection. The most intensively studied laboratory strains, e.g., the mouse leukemia viruses, are of this type. They were especially selected to be virulent, easy to grow, and to produce cancer readily on injection into animals. It now appears that most wild type viruses as they are found in natural animal populations are not of this variety but are endogenous in origin. This means that these viruses can exist as an integral, heritable component of the cell genome. The viral genome is normally present in the cell in an unexpressed state from which it can be activated spontaneously, or by a variety of artificial means.

Neither the endogenous nor the exogenous RNA viruses are contagious in the usual sense. However, recent experiments with the feline leukemia virus (FeLV) have shown that this virus can spread from animal to animal especially when the animals live in crowded conditions. Gibbon ape leukemia virus also appears to be able to spread in laboratory colonies as does avian myeloblastosis virus. The mechanism of transfer of viruses between animals is not known, but it should be pointed out that the rate of spread observed is extremely low and rare as compared to diseases normally described as contagious.

Endogenous viruses have been recovered from cells of mice, rats, hamsters, cats, chickens and baboons; all clones from apparently virus-free cultured cells of these species can be induced to yield complete or partial expressions of type C viruses. Some endogenous viruses have difficulty in replicating efficiently in the cells of the species of origin, but preferentially replicate in cells of other animal species; thus a subclass of endogenous viruses now called xenotropic has been recognized. The fact that endogenous viruses are at least partially expressed when the cells which harbor them become malignant indicates that their study is important. Many investigators now accept as a working hypothesis that natural RNA tumor agents are of endogenous origin.

Much of the recent evidence that RNA tumor viruses exist in unexpressed form is derived from studies in inbred mouse strains. Genetic and biochemical evidence indicates that multiple copies of the viral information are integrated within the DNA of the cell. Endogenous viruses are activated spontaneously during long-term passage of cells in tissue culture and by co-cultivation with cells of a different species. The induction rate can be increased 1000-fold by treatment with carcinogenic chemicals, x-irradiation, or infection by exogenous type C viruses. Halogenated thymidine analogs (IudR, BudR) and protein synthesis inhibitors (actinomycin D) are extremely efficient inducers, increasing the rate of expression by more than 1 millionfold. Endogenous viruses can also be induced by passage of tumor material from one animal species into another. This is especially true when the recipient species has been immunosuppressed. An example of this method

was the recovery of the RD-114 virus after injection of human sarcoma cells into the brain of a fetal cat. Although this virus has antigens distinct from those of known feline type C viruses, hybridization studies revealed that the virus was a feline endogenous virus.

Several independent endogenous murine type C viruses have been isolated. Each appears to be of mouse origin as determined by the antigenicity of its major virion polypeptide, p30. Although at first these virus isolates were indistinguishable from each other in serologic and host range properties, newer findings have shown that they differ considerably in the immunologic type-specificity of their p12 polypeptide. Furthermore, some grow preferentially in cell lines of another strain. For example, a virus inducible from BALB/c cells grows to markedly higher titer in NIH Swiss than in BALB/c cell lines. It is thus possible to distinguish different subgroups of endogenous viruses from the same species of origin. As these studies continue, it is quite likely that many subclasses of endogenous type C viruses will be discovered.

The role of endogenous viruses in naturally occurring malignancies has yet to be elucidated but certain suggestive facts are known. One class of type C virus that is chemically inducible from virus-negative mouse cells in tissue culture can induce lymphatic leukemia in vivo. The type B mouse mammary tumor virus sometimes transmitted through the milk, as well as genetically, is an endogenous virus that causes mammary cancer. In high cancer incidence mouse strains the titer of endogenous virus in the serum early in life correlates extremely well with the frequency of malignancies in that strain. In addition to their role in cancer, there is evidence that endogenous viruses may be responsible for certain autoimmune diseases such as glomerulonephritis.

Ubiquity of RNA Tumor Viruses. Viral DNA sequences related to those of RNA tumor viruses have now been demonstrated in the normal cells of many vertebrate species including primates and man. Indeed, it is generally believed that viral genes can be found in all normal vertebrate cells where they probably have an important, though as yet unknown, function in normal development. By means of a series of hybridization experiments it was shown that these ubiquitous viral sequences have probably become part of the animal genomes many millions of years ago and have evolved with their host species during the course of evolution. Today the relationship of the endogenous viral genes with each other parallels closely the taxonomic relationship of the animals in which they reside.

Control of RNA Tumor Virus Expression. The presence of an RNA tumor virus in an animal does not necessarily lead to the development of malignant disease. Numerous genetic and environmental factors influence the expression of the virus; both viral and host genes are known to be involved.

The genetic effects of the host have been intensively studied in inbred strains of mice. During the last few years several host genes affecting viral function have been identified and the chromosomal location of some of them determined. A better knowledge of the mechanism of action of these genes may provide us with a means for controlling tumor viruses artificially.

The genetic makeup of the virus also influences the rate of tumor formation. This clearly follows from the observation that different viral strains produce different types and degrees of disease in the same animal. Many investigators are attempting to identify the viral gene or the viral product that will cause a susceptible cell to become malignant. Better tools for attacking this problem are becoming available all the time. We know now that viruses can lose the ability to cause malignant transformation by mutation, but the biochemical nature of the "transforming principle" is still a mystery. Knowledge of the nature of this "transforming principle" may be a key to the understanding of transformation of cells to malignancy.

Structure of RNA Viruses. The RNA tumor viruses consist of a core of RNA and some proteins, including the enzyme RDDP, and an envelope composed of a variety of proteins and glycoproteins with which the virus becomes coated as it passes through the cell membrane. Basically the type C and type B viruses are quite similar in composition. The following discussion will, therefore, be mainly about type C viruses with reference to type B viruses where these differ significantly.

Viral RNA. When RNA is extracted from type C viruses the major components sediment at 60-70S and at 4-5S. The 60-70S RNA can be denatured into subunits sedimenting at 30-40S. From the molecular weight of the subunits one can estimate that between 2 and 4 such units make up the 60-70S viral RNA. When examined under the electron microscope, two subunits appear to be linked together by multiple bonds. According to the latest experiments the subunits found in one virus are all identical and have a molecular size of about 3×10^6 daltons. Comparison of the RNA subunits of leukemia and sarcoma viruses shows that they are of different length but have certain sequences in common. It appears that avian sarcoma virus RNA consists of complete leukemia virus RNA molecules which have picked up additional RNA sequences that presumably give the virus the ability to transform cells. In the case of murine sarcoma viruses, which are replication-defective, both a loss of some leukemia virus RNA sequences and a gain of new RNA sequences appears to have occurred.

The small RNA components found in the virus are probably of cellular origin. One of them acts as the primer necessary for transcription of viral RNA into DNA by RDDP.

Viral RDDP. One of the most characteristic properties of RNA tumor viruses is that they contain an enzyme which specifically transcribes viral RNA into DNA. The enzyme is clearly different from cellular DNA polymerases in its properties and in genetic experiments has been shown to be coded for by a viral gene. Type B viruses have a polymerase that differs in cation requirement from that of type C viruses and can, therefore, be readily distinguished biochemically.

RDDP of avian RNA tumor viruses has a molecular weight of 70,000 and consists of two subunits; one has the enzymatic activities associated with the enzyme and the other appears to be required for binding to the template. Since the

product of the synthetic reaction catalyzed by RDDP is small pieces of DNA, other enzymes are needed in addition to RDDP to form the high molecular weight DNA that is the final product in the cell.

It is not clear how mature viruses are produced from the DNA copies made by the viral polymerase, but it is believed that DNA synthesis is an obligatory intermediate step. The DNA copies are also necessary for the integration of the viral genome into the host genome: this may be another obligatory intermediate step in viral replication. Although the mechanism of viral integration is not known, it has been shown that transformed cells contain more viral DNA copies than untransformed cells. Viral genomes can thus become part of the host cell genome, as well as become independent of it again.

Viral, Virus-Associated and Tumor-Specific Antigens. In recent years several structural proteins of RNA tumor viruses have been isolated and characterized. The purified proteins and glycoproteins have been used to produce highly specific antisera and to develop radio-immunoassays which are more sensitive than standard serologic tests. These reagents have proven valuable for the characterization of new viral isolates, for the study of viral gene expression, and in the search for possible oncogenic viruses in human neoplasia.

Table 1 summarizes our knowledge of viral structural proteins to date.

Table 1
Major Structural Antigens of Type C Viruses

Protein	Location in virus	Specificity
gp 70 (69-71)	envelope	type, group, inter
p 70	core	group, inter
p 30	core	type, group, inter
p 15	envelope	type, group, inter
p 12	envelope	type, subgroup
p 10	core	group

A new nomenclature has been adopted describing the proteins by their molecular weight and indicating whether they are proteins (p) or glycoproteins (gp). Three of the known proteins are located in the core of the virus particle and three in the envelope. All the proteins have several kinds of immunologically reactive sites: type-specific determinants that do not crossreact with those of any other virus; group-specific determinants common to all viruses of one species of host animal; interspecies determinants common to viruses from many different host species. Some viral proteins have mainly group-specific determinants which are useful for classifying the virus according to species of origin. Other proteins have mainly type-specific determinants and are, therefore, useful for differentiating between the viruses of one species. The two type C components most prominent in the virus structure are the major envelope glycoprotein gp70 (5%) and the major

core protein p30 (30%). Both possess interspecies determinants which cross-react with viral proteins of different species. Antisera produced against gp70 possess strong virus neutralizing activity.

Type C virus-induced tumors contain three major groups of virus-related antigens: viral structural antigens, antigens coded by the viral genome but not structural (virus-associated), and cellular genome-coded antigens expressed as a result of malignant transformation (tumor-specific). A variety of cellular control mechanisms determine which of these antigens (and to what extent) are expressed in each cell harboring a virus. Antigens of each group can be expressed on the surface of the cell, a fact which is important to any consideration of viral control because the immunological defense mechanisms of the host readily attack cell surfaces. Table 2 summarizes our present knowledge about the expression of these antigens on the cell surface.

Table 2
Antigens on Surfaces of Virus-Infected
Nonmalignant as well as Malignant Cells

Characteristics of CSA	Presence
CSA determinants common to VEA	Proven
CSA determined by the viral genome	Proven
CSA determined by the cellular genome	Partially proven
Derepression of fetal antigen	Proven

CSA (cell surface antigen); VEA (viral envelope antigen).

There is now considerable evidence that gp70 is a virus gene product and is expressed on the surface of transformed cells even in the absence of other virus expression. Its accessibility to antibody at this site has been demonstrated by a variety of immunologic techniques. Cell surface determinants resembling those of gp70 have also been identified on nonmalignant and leukemic lymphocytes and thymocytes of certain mouse strains and in normal and neoplastic human cells.

The evidence for the surface expression of other viral antigens is not as convincing. The recent demonstration that antiserum to p30 is cytotoxic in the presence of complement for cultured mouse tumor cells suggests that this antigen is also on the cell surface. A similar finding has been reported for p10. The presence of these internal components at this cell site is somewhat surprising since p30 has only rarely been detected on the surface of mouse lymphoma cells which contain large amounts of this antigen. A distinct possibility is that p30 and p10 are adsorbed to certain cells as the result of disruption of other cells and/or virus disintegration. As yet, there are no reports that the other less prominent structural antigens of the virus are found on the cell membrane.

The most striking feature of virus-associated cell surface antigens (CSA) is that the tumors induced by a given virus, even in different species, share a common CSA. Different viruses, however, induce CSA with different specificities. This feature of common CSA specificity is of great advantage to

immunotherapy of virus-induced tumors. Virus-induced tumors also appear to have tumor-specific antigens, that is, antigens carried by all malignant cells of a certain kind regardless of the route by which they became malignant. The difficulty in disentangling the nature of all the cell surface antigens on malignant cells stems partly from the fact that all cells harbor endogenous viruses. These may be producing some proteins that appear on the cell surface but since in most cases the endogenous viruses have not been fully characterized, the characteristics of their proteins are also unknown.

An important aspect of the study of viral proteins is the knowledge of how the host responds to the antigenic stimulus of these proteins. Immune responses to virus and/or virus-associated antigens, especially to those found on the cell surface, could affect the fate of the host. For example, neutralizing antibody to certain determinants on the virus can inhibit viral activity, thus affecting cell-to-cell transmission of the agent and possibly oncogenesis; or lytic antibody reacting with surface components can destroy tumor cells. Such host responses can be modified by specific and non-specific immunization.

In the mouse systems there are many reports on the natural occurrence of antibodies to internal and envelope components of type C viruses. Because of antigen excess, the antibodies were found mainly in the form of immune complexes in the glomeruli of the kidney. More recent studies show that mouse strains with low incidences of spontaneous lymphoma produce antibodies to murine leukemia viruses. Free antibodies in the sera of these mice recognize proteins of the viral envelope.

In the cat, infection with feline leukemia virus (FeLV) appears to be fairly common and neutralizing antibodies to gp70 can be detected in the sera. A similar result has been reported for infections by murine leukemia virus (MuLV) and gibbon leukemia virus (GaLV) systems. The findings correlate well with the proposed horizontal spread of FeLV, MuLV and GaLV. Cats, gibbons and mice also produce antibodies to their homologous p30 antigens.

Thus, despite the persistence of these viruses throughout the animal's lifetimes, most species seem to be able to respond immunologically to type C virus antigens. As yet there is little information to determine whether these natural antibodies benefit the host, i.e., control virus expression or oncogenesis.

RNA Viruses: Relation to Human Cancer

The evidence that RNA viruses cause at least some animal cancers is very convincing. More species are continually being added to the list of those in which tumor-producing RNA viruses have been identified and there is good evidence that genetic material very closely related to RNA tumor viruses is present in all vertebrates. Since some of man's closest relatives have virus-produced cancers. there is every reason to believe that viruses cause cancer in man also.

The ease with which viruses can be isolated from a given species of animal varies markedly from species to species. The mouse releases viruses very readily: man has so far been one of the most difficult animals in which to find viruses. However, as new and more sophisticated procedures have come into use, previously unsuspected viruses have been uncovered.

Many of the known animal viruses grow well in human cells and can also transform them. When a virus is found growing in human tumor cells there is, therefore, always the problem of eliminating contamination by animal viruses. Since it is not possible to inject a virus into humans to test its oncogenic potential, other criteria have been developed for classifying viruses as human. There is, however, no clear-cut test for "humanness." Any human virus would be expected to be closely related to primate viruses and distantly related to other mammalian viruses in terms of nucleic acid homology and antigenic determinants. As we now know that the RNA viruses, including the proviruses in the cellular genome, can readily undergo genetic recombination with each other resulting in viruses that are of mixed origin, it is always necessary to test several properties of an unknown virus before classifying it. The question also presents itself: how much of a virus has to be human to call it human? Defective viruses are known, e.g., mammalian sarcoma viruses, which can only form complete viral particles with help from other viruses. The final particle of a defective virus can contain components belonging to its helper virus and could, therefore, give misleading results when tests for its origin are conducted. As more is known about the behavior of RNA tumor viruses the question of their natural host becomes more complicated. For example, RD-114, an endogenous cat virus, has nucleic acid sequences related to both cat and baboon DNA and p30 closely related to the p30 of an endogenous baboon virus but unrelated to p30 of other cat viruses. The curious properties of RD-114 can be explained if, millions of years ago, a primate virus infected an ancestral cat species from which the modern domestic cat evolved.

Evidence for Human RNA Tumor Viruses. A variety of evidence has accumulated suggesting that human cells, like animal cells, contain RNA viral material and that this is involved in the initiation of malignant growth. The recent isolation of viruses from primates, man's closest phylogenetic relatives, argues strongly that man also harbors such viruses. As discussed above, DNA related to endogenous RNA tumor viruses has been shown to be present in normal human cells. Some investigators are also finding that normal cell membranes of several animals and of man contain substances that crossreact immunologically with a well-defined viral glycoprotein, gp69-71. The significance of the latter finding is not understood and confirmation of the validity of the results is needed before conclusions can be drawn.

In the normal human cell, the viral DNA sequences appear to be largely repressed. If they have anything to do with cancer they should become activated when the cell undergoes transformation to the malignant state. The malignant cell should, therefore, contain appreciable amounts of typical products of RNA virus genes: RNA and protein molecules similar to those of the virus as well as the viral enzyme RDDP. Experiments to look for these

components in human tumor cells have been done and positive results have been obtained in some cases. The best studied examples are human breast cancer and human acute myelogenous leukemia (AML).

Normal human milk and cells from malignant human breast tumors contain biochemically defined "particles" that contain 60-70S RNA and RDDP. In human milk a few particles that look like type B RNA viruses have also been seen under the electron microscope. The RDDP is like the viral enzyme in its substrate requirements and is unlike any normal cellular DNA polymerase. The RNA of the milk "particles" has sequence relationship to the RNA of breast cancer cells and the RNA found in breast cancer cells, in turn, has sequence homology to the RNA of MMTV and MP-MV. Benign breast tumor tissues do not have particles, RDDP or RNA homologous to MMTV or MP-MV. Recently it was reported that a human breast cancer cell line was able to produce viruses at a very low level. This has been difficult to reproduce as the conditions required for virus production are elusive.

The case for viral involvement in human AML appears even more convincing. "Particles" containing 60-70S RNA and RDDP have been found in the leukemic cells of AML patients. The enzyme has been purified and studied extensively and found to be very similar immunologically to RDDP from two primate viruses--gibbon ape leukemia virus (GaLV) and woolly monkey virus (SSV). Hybridization studies showed that the RNA of the "particles" was closely related to the RNA of the same two primate viruses and more distantly to mouse leukemia virus RNA. As techniques became more refined it was possible to show that the human leukemia "particles" were more closely related to SSV than to GaLV or to any other virus tested. In all of five cases tested, human AML cells have also been shown to contain p30 antigens specifically related to those of the SSV-GaLV group. The p30 proteins of this group of viruses cannot be distinguished immunologically.

Several cell lines developed from blood samples of one patient with AML have produced intact viral particles after prolonged growth in vitro. The particles are seen to bud from the cell membrane under the electron microscope A factor from embryo cultures is required for the growth of these leukemic cells in vitro and the production of virus from them. The virus that is produced, like the "particles" found in other AML patients, is very similar to SSV in its antigenic specificity and nucleic acid sequences, and is less closely related to GaLV. The relationship of the AML virus to woolly monkey virus is so close that the possibility of the two viruses being identical cannot be completely ruled out. The woolly virus was obtained in California from a pet woolly monkey with fibrosarcoma and it is the only isolate obtained from this species of monkeys so far. It produces sarcomas and brain tumors in marmosets. In terms of the usual criteria of nucleic acid homology between virus and host DNA, the virus is not a bona fide woolly monkey virus, nor does it appear to belong to any other species tested. Even if the AML virus is identical to the woolly monkey virus, the possibility that it has something to do with the human disease cannot be dismissed without further study.

DNA Viruses and Human Cancer

The last ten years have seen the emergence of a large body of evidence linking the etiology of several human tumors to DNA viruses, especially those of the herpesvirus group. Prior to that time, the only evidence connecting herpesviruses to animal neoplasias was the finding of herpes-like particles in cells of the renal adenocarcinoma of the frog. The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's lymphoma in 1964 stimulated extensive studies to examine herpesviruses as possible etiologic agents of cancer in several animal species, including man. Herpesviruses recovered from wild cottontail rabbits, chickens, and higher primates are now known to be causally related to lymphoproliferative neoplasias in their respective hosts. These and other animal models have provided valuable experimental systems in which to study DNA virus-induced oncogenesis. At least two herpesviruses have been implicated as possible etiologic agents of certain types of human cancer--EBV with lymphoma and nasopharyngeal carcinoma, and Herpes simplex type 2 virus with squamous cell carcinoma of the uterine cervix.

Two types of virus-cell interactions have been recognized following exposure of susceptible cells to members of the herpes group of viruses. Productive infection results in an inhibition of host cell DNA, RNA and protein synthesis, and a concomitant accumulation of virus-specific components. The net result of this interaction is the synthesis of infectious progeny virus and destruction of the host cells. Therefore, cell transformation and productive infection by herpesviruses would, of necessity, be mutually exclusive processes. In fact, oncogenic conversion by herpesviruses appears to result from a nonproductive (abortive) infection in which viral functions responsible for inhibition of host cell macromolecular synthesis are not expressed. Stimulation of cellular DNA synthesis, acquisition of virus-induced antigens, incorporation of viral nucleic acids and transformation of normal cells into established cell lines have all been described in non-productive infections. Activation of the synthesis of infectious virus by exposure to halogenated pyrimidines or irradiation is usually accompanied by cell death. In vivo, herpesviruses also establish covert latent infections in which infectious virions cannot be recovered except during periods of overt disease. Whether latent herpesvirus infections are the result of low level productive or nonproductive interactions remains undetermined. Although horizontal transmission represents the usual mode of spread of these viruses in vivo, vertical transmission of the genome in nonproductively infected cultured cells has been recorded.

Epstein-Barr Virus. Evidence has been steadily mounting for an etiologic role of EBV in Burkitt's lymphoma (BL) of African children and, to a lesser extent, in nasopharyngeal carcinoma (NPC). The association between EBV and Hodgkin's disease, chronic lymphocytic leukemia, and various non-malignant diseases has recently been questioned since some patients were found to have no antibodies to EBV and the elevated EBV titers observed in others may reflect the effects of these diseases on the immunological containment of a usually self-limited EBV infection. Finally, it has now been firmly established that EBV is the causative agent of the heterophile-positive, classical

form of infectious mononucleosis (IM), a benign lymphoproliferative disease with predilection for adolescents and young adults.

The Epstein-Barr virus was discovered in the course of an intensive search for viruses associated with BL, a disease which is extremely rare outside of central Africa and parts of New Guinea. The climate-related geographical distribution of this highly endemic disease suggested that insect vectors played some role in its dissemination. The involvement of an infectious agent was strongly supported by the time-space clustering and epidemic drift of the disease. However, the relatively restricted geographical incidence of BL compared to the ubiquitous nature of EBV strongly suggested the interaction of this virus with other co-factors or predisposing conditions to induce this malignancy. A similar pattern is suggested for NPC since this tumor occurs mainly in adults and at an increased frequency in Southern China and certain regions of Africa where primary EBV infection commonly occurs very early in life.

Seroepidemiologic surveys to study the relationship of EBV to BL and NPC became feasible with the development of tests to detect antibodies to this virus in human sera. Patients were generally found to have elevated levels of antibodies to EB viral capsid antigens (VCA), cell membrane antigens (MA) and to the diffuse (D) or restricted (R) components of the EBV-induced early antigen (EA) complex. The spectra and titers of various EBV-related antibodies have been shown to be of diagnostic or prognostic value. For example, anti-EA activity is primarily directed against the D component in IM and Oriental NPC while antibodies to the R component prevail in BL and Caucasian NPC. Anti-VCA and EA titers are relatively low in the early stages of NPC and in long term survivors but increase gradually with progression of the disease. Similarly, an unfavorable prognosis for BL patients is indicated by a decline in anti-MA antibody production or an increase in anti-EA titer. Irradiation of BL and NPC in vivo has led to an increase of antibody titers against the EB virus-determined MA and VCA. Viral activation followed by antigen release or simple antigen release due to tumor disintegration may explain these effects.

The results of studies on BL and NPC tumor biopsies have been quite consistent. Virus particles, capsid and early antigens have been detected regularly in BL, but not NPC, tumors. Recently an EBV-determined nuclear antigen (EBNA) could be visualized by anticomplementary immunofluorescence in BL and NPC biopsies while an EBV-coded CF antigen, unrelated to VCA, MA or EA, has been measured in extracts prepared from BL tumors. The EBNA antigen shows a striking similarity with the T antigens in papova- or adenovirus-transformed cells. Multiple copies of EBV DNA have been demonstrated in virtually every BL and NPC biopsy and tumor cell line examined by molecular hybridization. Furthermore, recent experiments suggest the presence of EBV DNA in epithelial elements of nasopharyngeal carcinomas. This observation is especially important since only cells of the lymphoid series have been shown to undergo transformation by EBV in vitro.

Continuous lymphoblastoid cell lines have been established from BL or NPC biopsies, from peripheral lymphocytes of IM patients, and from normal

lymphoid cells exposed to EBV. In the latter case, transformation is prevented by prior exposure of the virus to neutralizing antibodies, to heat, or to ultrafiltration. Adult peripheral white cells may convert into these cell lines without the addition of extraneous virus, but the derived cells usually carry EBV. In contrast, cultures initiated from donors without EBV-directed antibodies or from cord blood or fetal organs fail to survive. The available data indicate that EBV is the sole or major determinant required for establishment of cell lines of lymphoid origin. Although the antigenic profile and virus productivity patterns vary among these cell lines, EBV-homologous nucleic acid sequences are always detectable. Virus expression can be enhanced in some cases by arginine starvation, x-irradiation, or small doses of various synthetic metabolic inhibitors. Evidently the virus genome persists in these cells, is expressed in varying degrees, and, at least in vitro, is transmitted to progeny during cell division.

Recent efforts to demonstrate the oncogenic potential of EBV in vivo have met with notable success. Fatal tumors resembling reticulum cell sarcomas were observed in marmosets injected with IM-derived EBV which was propagated in simian cells. In addition, a fatal lymphoproliferative malignancy developed in an owl monkey following injection of cells from an EBV-producing culture of BL origin. If these results can be confirmed and shown to be related to the presence of EBV in the respective inocula, at least two animal models will become available to study the oncogenicity of EBV.

Although these observations strongly support an etiologic relation of EBV to African BL and to NPC, its role could be that of an accessory factor or passenger virus because high anti-EBV antibody titers are not always detected in BL patients and tumors of the Burkitt type do occur in temperate regions. In fact, a considerable proportion of European or American BL patients failed to show a serological association with EBV. In addition, no EBV DNA has been found in American BL tissues even though this tumor is histopathologically similar to African BL. RNA sequences homologous to the genome of a murine leukemia virus have been identified in BL and NPC biopsies suggesting the possible interaction of a type C virus and a herpesvirus in the genesis of these diseases. In this regard, biopsies of two recent African BL patients failed to reveal EB viral DNA and EBNA, but another nuclear antigen was demonstrated in the tumor cells by the anti-complement immunofluorescence technique using sera from patients with acute myelogenous leukemia (AML). The possibility that this leukemia-associated nuclear antigen (LANA) marks the presence of a human RNA tumor virus is supported by the recent isolation of a type C virus from the blood cells of a patient with AML. If we assume that infection by EBV primes the cell for neoplastic change, it is possible to conceive that one or more combinations of environmental and host factors could interact to promote oncogenesis. Thus, the association of EBV infection with more than one neoplastic disease becomes more plausible. Should this virus prove to be a necessary co-factor in the induction of disease, control of infection will be extremely important.

Herpes Simplex Virus. Carcinoma of the uterine cervix is now clearly recognized as the second most common malignant disease of women in the United States. Epidemiologic studies provided the first suggestive evidence

that an infectious, venereally transmitted agent was associated with this disease. Later cytohistopathologic, virologic, and seroepidemiologic studies confirmed this observation and identified the suspect agent as Herpes simplex virus type 2 (HSV-2). It is now clear that squamous cell carcinoma of the cervix is related to sexual activity. The pivotal demographic and epidemiologic characteristics that distinguish women at greater risk to developing cervical cancer are low socioeconomic status, early age at first coitus, and number of sex partners.

Seroepidemiologic studies have provided the strongest evidence associating HSV-2 with cervical cancer. Generally, antibodies to HSV-2 have been found more frequently and in higher titer among women with cervical cancer than among control women of similar age, race and socioeconomic level. The antibody activity was found to be age-dependent among control women but not among women with cancer, suggesting that women in the latter group are infected by HSV-2 earlier in life. These differences between patients with cervical carcinoma and controls are more impressive in Black than in Caucasian women and in the U.S., Belgium, and Denmark than in Israel, Columbia, and New Zealand. The occurrence of antibodies to HSV-2 has been noted to increase with progression of the disease; the lowest incidences were found among women with dysplasia and the highest among women with invasive cancer.

Some inconsistencies have been observed in the seroepidemiologic studies and may, in part, be related to shortcomings in the serological specificity of the tests employed. The accuracy with which the present assay methods detect past infections with HSV-2 is unknown. Herpes simplex virus types 1 and 2 appear to share common antigens, and, in addition, have one or more type-specific antigens. Infection with either virus results in the production of antibodies that will crossreact with the heterotypic virus. A prior infection with HSV-1 modifies the production of antibodies to HSV-2 while an infection with HSV-2 in an individual previously infected with type 1 virus may stimulate an anamnestic response to the shared antigens of the two viruses. Thus, the criteria used for assessing positivity or negativity for antibodies to HSV-2 may not adequately depict the status of a past infection with this virus. Clearly, the isolation, purification, and characterization of HSV type-specific antigens is crucial to definitive seroepidemiologic studies.

HSV-2 nonvirion antigens have been described in cells infected in vitro with this virus. Antibodies to these antigens have been detected in the sera of a high percentage of cervical cancer patients. Additionally, soluble membrane antigens extracted from cervical tumors were shown to react with antibody directed against HSV nonvirion antigens. One of these nonvirion antigens, AG-4, appeared in cultured human cells four hours after infection with HSV-2. Antibody to this antigen was found almost exclusively in women with cervical neoplasia, displayed a pattern of increased prevalence in patients with more advanced stages of the disease, and was absent in women who had undergone successful tumor therapy.

Infectious virus, viral structural antigens, and HSV-specific cytoplasmic changes have not been detected in cervical cancer biopsies. However, virion structural antigens were observed in exfoliated tumor cells and tumor cells on the periphery of neoplastic lesions. Further evidence for the persistence of the HSV genome in cervical tumor cells was gathered from in vitro experiments in which infectious virus and viral antigens were detected in spontaneously degenerating cell cultures derived from a carcinoma in situ. Changes similar to those appearing spontaneously could be induced by exposure of the cells to medium of high pH. Virus expression in a culture derived from an invasive cervical carcinoma was limited to a membrane fluorescence on 2% of the cells which could not be augmented by spontaneous or high pH-induced cell degeneration. This data suggests that some cervical cancer cells harbor the complete HSV-2 genome in a repressed state and virus expression occurs following exposure of the cells to conditions of stress.

Photodynamically-inactivated or UV-irradiated HSV-2 has been shown to transform normal hamster embryo fibroblasts in vitro. A variety of serologic tests have yielded evidence for HSV-specific antigens in the cytoplasm and on the surface of transformed cells. These cells were tumorigenic when inoculated into newborn hamsters and induced virus-neutralizing and membrane reactive antibodies in the sera of tumor-bearing animals. Pre-immunization of hamsters with HSV failed to induce transplantation immunity but instead enhanced tumor metastases while immunization with SV40 inhibited metastases but did not reduce the rate of primary tumor development. Transformation in vitro of hamster cells by UV-irradiated HSV-1 and cytomegalovirus and of human embryonic lung cells by heat-inactivated HSV-2 have also been reported.

These findings strengthen the relationship between HSV-2 and cervical carcinoma, but further studies are needed to determine the exact role of this virus in cervical cancer. If HSV-2 can be shown to play some essential part in the induction of this disease, appropriate control measures may be developed to reduce the incidence of this human cancer.

Papovaviruses. Polyoma virus, SV40 and the papilloma viruses are oncogenic members of the papova group of viruses. The capacity of these agents, particularly polyoma virus and SV40, to produce malignant tumors in several different animals is well known. In addition, SV40 has been shown to transform morphologically a variety of cultured cells, including those of human origin. During the past three years, several papovaviruses of the polyoma-SV40 group have been isolated from humans. These viruses can be classified into three antigenically distinct groups which crossreact to some extent with SV40 but not with polyoma virus. Since seroepidemiologic evidence has been obtained indicating the widespread colonization of human populations by this group of viruses, their role in human neoplasia is being systematically examined.

Treatment and Control

The most impressive evidence for the viral etiology and perhaps the best hope for prevention and control of cancer would be the demonstration that anti-viral agents or anti-viral immune responses can prevent or modify the disease. There are a number of reasons for attempting to use the information now available on RNA tumor viruses in the control of cancer. (a) The oncogenicity of these viruses in vertebrate species is well-established. (b) In contrast to chemically-induced tumors, cells transformed by a given virus share common antigens, which are determined by the viral genome, either as virus structural antigens and/or as virus-associated antigens. (c) Several antigens have been isolated, purified and characterized with respect to the serologic reactivities directed against them. Some of these are expressed at the cell surface whether or not virus particles are produced. (d) Naturally occurring antibodies to type C virus components and virus-associated antigens have been demonstrated in the animal host. While the correlation of the immune responses with the incidence of malignancy needs to be determined, active or passive immunization may be an approach to the control or prevention of cancers of animals and man.

Immunologic Treatment. Nonspecific stimulation of the host's immune response can be accomplished by a number of chemical agents. Some of the agents that stimulate the reticuloendothelial system nonspecifically, e.g., imidazolethiazole, pyran copolymers, tilorone, BCG, etc., have proven effective in increasing the survival time of leukemic mice. Levamisole has proved expecially effective when used in combination therapy and is presently being used in preliminary clinical trials.

Specific stimulation of the host's immune response is achieved by vaccination with viruses, viral antigens or virus-associated antigens. It now appears possible to use virus vaccine-induced antibodies to modify natural type C virus expressions and either delay or prevent tumors associated with these viruses. Early experiments established the feasibility of using formalin-killed virus vaccine in the prevention of murine leukemias induced by infection with the Rauscher strain of MuLV. Since spontaneous virus-induced cancers appear to be caused by endogenous viruses which are present in the animal from the moment of conception, the prevention of malignancy by these viruses is not as straightforward as the prevention of diseases caused by viral infections. However, with some notable exceptions in highly permissive animal systems, most spontaneous cancers in natural species occur late in life. On the other hand, effective virus-specific neutralizing and cell-mediated immune responses to endogenous viruses can be produced early in life by live, attenuated or formalin-killed viral vaccines. Therefore, prevention of spontaneous cancers by viral vaccines now appears feasible. Studies to test such vaccines are underway in a number of laboratories but since such studies are of necessity long-term final results are not available at this time.

Any vaccine containing live or inactivated oncogenic viruses has definite disadvantages. There is always the possibility that the viral RNA, alone or in combination with other information in the cell, still has the capacity

to transform the cell. In order to avoid this potential hazard, investigators are studying the possibility of using viral antigens free of viral RNA in vaccines. Such antigens can be prepared either by purifying viral proteins or by using preparations of cell membranes that carry viral or virus-associated antigens. Both possibilities are being pursued. The disadvantage to this type of vaccine is that since proteins cannot replicate after injection into an animal as viruses do, a much larger amount of material has to be used to obtain an equivalent immunological response. In man, vaccines of this type would probably have to be limited to high risk groups.

In this regard, it is interesting that homogenates of tumor cells infected with certain non-oncogenic viruses are more immunogenic than extracts of non-infected cells. For example, extracts of SV40-transformed cells infected with influenza virus or VSV produce much better immunity in mice than do uninfected cell extracts. Viruses which seem suitable for the purpose of "virus-assisted immunotherapy" in man have been adapted to human malignant tissues and a new research strategy towards human application is underway.

Neuraminidase treatment of tumor cells has also been found to increase their effectiveness as immune stimulators. The injection of neuraminidase-treated tumor cells greatly increases the lifespan of AKR mice undergoing chemotherapy. Such cells are now being used in the treatment of patients.

Biochemical Treatment. A number of compounds that specifically inhibit viral functions have been identified. Some compounds, such as colcemid and fluorodeoxyuridine, increase the reversion frequency of certain transformed cells to the normal state. Other compounds can specifically inhibit the viral RNA dependent DNA polymerase. Interferon, an anti-viral agent, has been found effective in reducing cancer incidence in animals receiving grafts. None of the chemicals so far tested promises to provide a magic cure for cancer, but some have proved useful in combination with other treatment.

b. Progress Highlights (not covered in Narrative)

Type C RNA Viruses

Radioimmunoassays using antiserum to the RD114 cat virus and RLV p30 antigen detect a class of interspecies determinants common to murine, feline and non-human primate viruses. Antigen with the same apparent affinity for antibody as that of the interspecies determinants of the highly purified p30 RLV core protein, was found in relatively high concentrations in the tissues from patients with systemic lupus erythematosis, and in lower concentration in some neoplastic tissues and in normal human tissues. The competing protein extracted from the spleen of a lupus case, chromatographed on phosphocellulose, was similar in gel filtration characteristics to known p27 - p30 viral proteins.

The cytotoxic action of a monospecific antiserum for FLV gp71 against human myelogenous leukemia cells suggested the presence of cross-reacting cell surface antigens. The cytotoxic reaction could be blocked by gp71 and by the positive target cells but not by normal or lymphatic leukemia cells.

Cytoplasmic particulates recovered from quantities of acute myeloblastic leukemia cells contain a reverse transcriptase activity, but do not resemble virions morphologically. The reverse transcriptase from these particulates was shown to exist in two interconvertible forms of molecular weights 70,000 and 150,000. Since the two forms differ in their biochemical characteristics, interconvertibility may be associated with some regulatory function of polymerase activity.

Studies on terminal deoxynucleotidyl transferase in humans have shown that this enzyme is an extremely good marker for cells from acute lymphoblastic leukemia. Normal people have the enzyme only in their thymocytes and, at low levels, in the bone marrow. Three forms of the enzyme have been identified: two forms which are found both in thymocytes and in acute lymphoblastic leukemia cells and one form which has thus far been found only in the bone marrow.

By several criteria, including nucleic acid sequence homology and antigenicity of the polymerase and the p30 protein, the various baboon viruses are related to, but distinct from, the endogenous cat viruses of the RD-114/CCC group. The unexpected relatedness of the endogenous cat and the endogenous baboon viruses suggests that they have a common origin. Although all members of the cat family Felidae are closely related as shown by hybridization of their cellular DNA, only four species of the genus Felis (domestic cat, sand cat, and European wildcat) contain endogenous virogenes related to the RNA of RD-114. However, all Old World monkey and ape species examined have nucleic acid sequences related to RD-114. These findings suggest that viral genes from primates gave rise to infectious particles that integrated into the DNA of an ancestor of the domestic cat and have since been transmitted as cellular genes. By examing the divergence time of the various species discussed above, this infection is believed to have occurred about 5-7 million years ago in the Mediterranean basin.

Antigens related to the major structural protein (p30) of baboon type C viruses have also been detected in normal baboon, rhesus monkey, and stump-tail monkey tissues, and more recently, in several human tumors. These results indicate that humans, like other primates, contain endogenous type C virogenes in their DNA which can, in certain circumstances, be at least partially expressed.

It is characteristic of endogenous type C viruses that all individual animals of a species and all tissues of each animal contain nucleic acid sequences in the chromosomal DNA that code for the production of complete type C viral particles. Each species contains multiple (5-15) copies of endogenous virogene sequences per haploid genome, but heterologous species infected with and producing high titers of these type C viruses contain fewer copies (1 or 2 per haploid genome). The presence of multiple copies of viral information is thus a useful criterion with which to identify the species of origin of an endogenous mammalian type C virus.

More evidence has accumulated showing that viruses passaged through heterologous species lines, however briefly, pick up not only nucleic acids but also proteins of the new species. Thus, hamster viruses passaged through mouse and rat lines contain not only rat, mouse and hamster RNA sequences but also mouse, rat and hamster viral proteins.

The Kirsten and Harvey strain of sarcoma virus (which were each isolated by passage of MuLV in rats) contain genetic information not present in either the Kirsten strain of leukemia virus or the Moloney strain of leukemia virus. The additional information present in the Kirsten and Harvey viruses comes from rat cells and is present in normal rat cellular DNA. Apparently, the rat genetic information present in the Kirsten virus is the same as that in the Harvey virus, but it is unresolved whether the new rat genetic information is of cellular origin or derives from a distinct type C virus in rats different from the known rat type C viruses.

The gibbon virus was shown to cross-hybridize with hamster viruses in addition to its previously reported cross-hybridization to mouse viruses. This raises the question of whether the gibbon virus and the woolly monkey virus are not examples of viruses which have undergone reassortment under natural conditions.

Analyses of the RNA of viral recombinants led to the conclusion that genetic recombination among avian RNA tumor viruses involves molecular crossing over.

Four new genes affecting virus replication and tumor incidence have been described in the mouse, thus providing more evidence for the critical role of genetics in cancer.

Soluble extracts of mouse cells with Fv-1^n or Fv-1^b genotype inhibit infection by B or N-tropic mouse leukemia viruses respectively. The agent responsible appears to be an RNA molecule that affects some early step after penetration.

The stimulation of Type C virus production by dexamethasone and inhibition by interferon was shown to be a post-translational effect on the gene expression of RNA tumor viruses. It was found that this treatment did not alter the amount of intracellular viral protein, suggesting that dexamethasone and interferon act at the step after the viral proteins are synthesized, perhaps at the assembly of virus particles.

Most cellular DNA polymerases from several sources (e.g., nonactivated AKR cells, normal mouse spleen) have no affinity for poly(Um)-Sepharose columns at low salt concentrations. Reverse transcriptase (from RLV, AKR virus, activated AKR cells, or RLV-infected spleens) binds to the column and can be eluted with an NaCl gradient, leading to separation of the viral enzyme from most cellular DNA polymerases.

NZB mice produce large amounts of endogenous xenotropic virus early in life and correspondingly large amounts of immune globulins which form antigen-antibody complexes. Such complexes interfere with normal functions of the kidney and bone marrow, thus simulating the lupus-syndrome in man. Azathioprine immunosuppression of NZB mice during early life leads to early lethal leukemias, lymphomas and sarcomas, a finding that demonstrates not only the importance of immune responses in the control of the cancer but also rather strongly suggests that the large amounts of the xenotropic virus present in the leukemic tissues may be the actuating cause of the leukemias.

Antibody-dependent lymphocyte cytotoxicity may play a role in immunity directed against tumors induced by the Moloney sarcoma-leukemia virus complex. The sera from mice in which tumors had regressed actively mediated the destruction of Moloney virus-producing cells by normal or immune lymphocytes. This activity was detected only in the sera from "regressor" mice.

The high incidence of neoplasia in human organ graft recipients stimulated related studies in murine systems. It was observed that Type C RNA virus was activated by the graft versus host reaction leading to the development of malignant lymphoma in as many as one-half of the animals. Treatment with interferon inhibited virus activation and prevented development of lymphomas. Immune suppression in animals receiving skin grafts from animals even with only minor histocompatibility differences considerably increased virus activation in comparison to either treatment alone.

Five chemicals and 3 biologicals capable of stimulating host immune responsiveness have been tested in mice for use as immunoadjuvants against several leukemia and carcinoma tumor systems in combined modality therapy studies. Of the 5 chemicals, levamisole has been examined in depth. The activity of this chemical immuno-adjuvant is expressed by stimulating the T-lymphoctye population. The effectiveness of levamisole as an immunoadjuvant in chemotherapeutically-induced remissions of leukemias and carcinomas has led to its incorporation into clinical protocols designed to treat human leukemia and colorectal adenocarcinomas.

Type B RNA Viruses

Three groups have independently shown that human leukocytes that are

reactive against human breast cancer cells are also reactive in migration inhibition assays against mouse mammary tumor virus but not against type C mouse viruses.

Radioactive DNA (^{3}H-CDNA) complementary to the RNA of Mason-Pfizer Monkey Virus was used in molecular hybridization experiments to demonstrate sequence homology between MPMV RNA and RNA of human malignant breast tumors. Hybridization products were analyzed by cesium sulfate equilibrium density centrifugation and hydroxyapatite chromatography. Twenty seven of thirty-nine human malignant breast tumors tested contained MPMV-related RNA while no sequence homology was found in RNA from human benign breast tumors or normal breast, spleen, liver or kidney.

Successful suppression of MuMTV expression and tumorigenesis by immunization with MuMTV has been accomplished. Two mouse strains, Af/C57BL and RIIIf/C57BL, were immunized with killed MuMTV in complete Freund's adjuvant. Expression of MuMTV up to the 10th lactation was significantly suppressed and tumor incidence at 15 months of age was reduced.

Infection and replication of MuMTV was obtained when a cat kidney cell line was exposed to the virus. No morphological difference was observed in the infected cells. Typical type B particles were recovered from supernatant fluids of cat kidney cultures serially passaged over 12 months. Virus production was also confirmed by membrane immunofluorescense.

A virus induced to replicate in guinea pig cells by exposure to halogenated pyrimidines has been shown to be related to the mouse mammary tumor virus. This close relationship has been demonstrated by similarity in ultrastructure and mode of replication, by identical density and by the presence of a reverse transcriptase preferring Mg^{++} instead of Mn^{++} in the reaction mixture.

DNA Viruses

A genetic contribution to the etiology of nasopharyngeal carcinoma (NPC) was indicated by studies in Singapore which showed that NPC patients and their relatives have an HL-A antigen on their leukocytes which is rarely detected in non-Chinese or in Chinese control patients.

Using tumor sections from nasopharyngeal carcinomas, *in situ* hybridization methods have provided strong evidence that EBV DNA is located in the malignant epithelial cells. Also, recently the carcinoma cells, freed of lymphoid cells by passage through "nude" mice, were shown to contain virus DNA and EBNA antigen.

When mouse cell cultures (JLSV-0) chronically infected with Rauscher murine leukemia virus were superinfected with HSV-1, the HSV progeny produced syncytial lesions on chicken embryo fibroblasts in contrast to the round cell plaques produced by the parental virus stock. The HSV variant is lighter in cesium chloride gradients than the parental stock and preliminary experiments show differences between the two DNAs. Of further interest is the ability

to isolate viable Vero cell clones after infection of the cells by the syncytium-producing virus whereas this has not been possible with HSV-1 parental stock. These clones offer an opportunity to determine which HSV DNA fragments may persist within the cells and may provide a DNA probe for the examination of malignant human cells for the presence of HSV genetic information.

Co-carcinogenesis

RLV antibody specifically protects against malignant transformation by 3-methylcholanthrene in a well established rat cell culture chronically infected with RLV. Not only is transformation prevented but the RLV in the chronically infected cells is reduced to almost undetectable levels. RLV infected but antibody untreated cultures are readily transformed by the co-carcinogens; the use of antisera to unrelated RNA tumor viruses failed to protect.

Use of RadLV in low dosage as a live virus "vaccine" in three experiments in C57BL/6 mice resulted in significant reductions in the incidence of subcutaneous and intramuscular fibrosarcomas induced by 3-methylcholanthrene.

Significant enhancement of transformation by chemicals and papovaviruses has been described in otherwise stable rat cells following derepression of endogenous rat type C virus expressions by IdU prior to chemical treatment. Endogenous xenotropic viruses can, therefore, also serve as a cocarcinogen.

5. Projections

The Viral Oncology Area set forth a long range research plan for the identification and control of virus-induced cancers of man. Many studies within the following broad categories have already been implemented and will be expanded in the coming year:

a. Virus (or Virus Expression)-Tumor Relationships

(1) Model Studies. Studies on animal RNA and DNA tumor viruses known to cause malignancies in several mammalian species will be continued. The results of these studies have already provided important information about tumor viruses that is applicable to the isolation and identification of human agents. Special emphasis will be given to determine the characteristics of several newly isolated primate viruses, since these agents may provide the best probes for detection of type C virus information in human cells. This work will remain an integral part of the Program.

(2) Human Studies. Efforts to identify viruses or detect virus expression in human tumors have been underway for some time. The Program will continue to increase its activities in the search for viruses which induce malignancies in man.

(a) To identify and isolate candidate viruses or subviral products in leukemias, lymphomas, sarcomas and carcinomas.

(b) To identify and isolate candidate viruses or subviral products in lung, colon and other carcinomas.

(c) To develop methods for the detection of high cancer risk groups, i.e. individual susceptibility or predisposition to transformation by human viruses.

(d) To extend existing and develop new methods to induce tumor virus expression in "normal" cells.

(e) To develop suitable reagents and to improve existing immunological and biochemical methods for mass diagnostic screening for candidate viruses.

(f) To characterize, biologically and biochemically, presumptive human virus isolates.

(g) To increase emphasis on understanding the relationship of environmental agents (e.g. chemical carcinogens) as co-factors in viral carcinogenesis.

b. Molecular Studies

Major progress in the understanding of the molecular pathways of tumor virus replication has been made within the past year. Such advances have already provided the basis for new, extremely sensitive methods for the detection of oncogenic viruses or virus expression. It is now possible to characterize agents detected in specimens of human cancer patients in terms of their content of high molecular weight RNA and of RDDP. Specific hybridization procedures already provide a method for further investigation of host-cell-virus relationships which have been extended into the study of human cancers. Preliminary results offer strong supportive evidence that certain human tumor cells contain genetic information related to that found in known oncogenic viruses.

(1) Basic Studies. The Program will continue to broaden its activities for detecting, identifying and characterizing the spectrum of enzymes and their products required by tumor viruses for replication and/or transformation.

(2) Applied Studies. As knowledge of the fundamental molecular events in virus-cell interaction increases, the Program will continue to apply this information to the study of human cancer as follows:

(a) To identify and characterize similar enzymes or enzymatic activities within normal and malignant human cells.

(b) To develop highly sensitive methods for the detection of virus or virus activity in human cells.

(c) To develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral replication and/or tumorigenesis at the cellular and subcellular levels. The therapy could be directed at any or all of the stages of cell transformation beginning with cell infection by a tumor virus.

Ultimately these approaches will require an intensified program to develop drugs, anti-enzymes, gene repressors, or inhibitors effective at the molecular level.

c. Immunological Studies

Immunologic research has provided extremely sensitive techniques for detection and characterization of tumor viruses, viral antigens, and changes in surface membranes of tumor cells. These studies have also contributed to an understanding of the role of immunological mechanisms in host-tumor and host-virus interactions which provide a rational approach to the prevention and treatment of cancer.

(1) Basic Studies. Investigations of selected model systems, representing tumors induced by type C, type B and herpesviruses, will be extended to further identify, characterize and determine the viruses, viral antigens, and membrane antigens of tumor cells. The studies include development and application of improved techniques to detect cellular alterations induced by tumor viruses alone or as the result of interaction with other environmental agents. Research on spontaneous or naturally occurring tumors in model systems relevant to human cancer will be continued as a basis for a rational approach to prevention and treatment:

(a) To study cellular and humoral immune mechanisms and to determine their relative significance in host recognition of and response to virus-induced tumors and/or tumor viruses.

(b) To develop methods to enhance host response to virus-induced tumors or tumor virus antigens.

(2) Applied Studies. As basic research provides the framework for identification and characterization of viruses, viral antigens, and virus-induced cell membrane alterations in human cancers, immunological methods will be applied:

(a) To relate candidate human viruses to known oncogenic agents.

(b) To identify and characterize intra- and interspecies viral antigens which are present in known mammalian tumors as probes for detecting human tumor viruses or viral antigens.

(c) To determine the presence of crossreacting antigens implying viral causation in various human tumors.

(d) To launch large-scale seroepidemiological surveys which will define populations at high risk to virus-induced cancers.

Clinical studies will be directed toward understanding and manipulation of immune mechanisms in human cancer as a basis for:

(a) Development of vaccines (viral or subviral components) from identified and fully characterized human tumor viruses.

(b) Determination of the role of host immune responses in virus-induced tumor recognition and rejection.

(c) Application of (1) and (2) in the prevention and control of human cancer.

d. Test Systems and Resources

(1) Test Systems. In vitro and in vivo (animal) test systems will be carefully selected to evaluate the work outlined in the previous research areas:

(a) To determine the oncogenic potential of candidate human viruses;

(b) To develop bioassay systems for testing viral and viral/chemical carcinogens;

(c) To begin viral vaccine (conventional or other) testing and immunization programs;

(d) To begin viral therapy testing programs;

(e) To explore special animal tumor systems, especially in primate species particularly relevant to human cancer;

(f) To develop and maintain well-characterized cell culture lines and animal stocks (small mammalian and primate species).

Many of these systems are being developed at the National Cancer Institute's Frederick Cancer Research Center.

(2) Resources. Since research efforts undergo continual change in emphasis and scope as new leads emerge, a variety of resources will have to be developed, maintained and coordinated:

(a) Human Resources - collection and storage of serum and tissue specimens; integration of data on clinical records, storage and distribution; computerization of specimen collection.

(b) Animal Models - maintenance of various mammalian animal colonies for basic research and special studies;

(c) Reagent Production - large scale production of animal tumor viruses for basic research; production of standardized lots of purified viruses; and production of high quality diagnostic reagents;

(d) Candidate Human Virus Production - intensive developmental research effort to isolate and produce human viruses;

(e) Biohazards Control and Containment - controlled environment facilities are required for research on known oncogenic viruses and candidate human tumor viruses as well as for maintaining animal colonies which are protected from extraneous infections.

6. Reports on Information Exchange Activities

International Agreements and Meetings Sponsored by the Virus Cancer Program (Fiscal Year 1975)

International Agreements

U.S.- U.S.S.R. Agreement on Health Cooperation
Franco-American Exchange Program

Meetings Sponsored by the VCP*

Ninth Annual Joint Working Conference - Virus Cancer Program.
Pacific Coast Tumor Virus Group (PACTVIGR).
Atlantic Coast Tumor Virus Group (ACTVIGR).
International Workshop on Production, Concentration, and Purification of Epstein-Barr Virus and Viral Antigens.
Symposium on the Characterization of Oncorna and Related Viruses.
Conference on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia.
Symposium on Immunologic Control of Virus-Associated Tumors in Man: Prospects and Problems.

Meetings Partially Sponsored by the VCP

Ninth Meeting on Mammary Cancer in Experimental Animals and Man. Pisa, Italy, October 28-30, 1974.
Second International Symposium on Oncogenesis and Herpesviruses. Nurenberg, Germany, October 14-16, 1974.

Meetings to be Partially Sponsored by the VCP

Third International Congress on Virology. Madrid, Spain, September 10-17, 1975.
VII International Symposium on Comparative Research on Leukemia and Related Diseases. Copenhagen, Denmark, October 13-17, 1975.

---

* Summaries of the major findings of these meetings are included below.

Participation in U.S.- U.S.S.R. Agreement. Developed within the framework of the US-USSR Agreement on Health Cooperation, a Memorandum of Understanding for further cooperation in the study of the microbiology, the immunology, and the molecular biology of cancer viruses was first signed on November 18, 1972. The Memorandum established procedures for joint studies through the exchange of information, materials and scientists.

Meetings held: November, 1972 - Moscow - US-USSR delegations;
November, 1973 - Bethesda - US-USSR joint subcommittee;
May, 1974 - Moscow - US-USSR delegations.

A U.S. delegation attended a joint Soviet-American Symposium on biology of oncogenic viruses of animals and man in Moscow in May, 1974. Visits were made to institutions actively engaged in the field of virology and immunology in Moscow, Sukhumi and Riga. Coordinator for the U.S. was Dr. J.B. Moloney, NCI; for the USSR, Prof. V.M. Zhdanov of the D.I. Ivanovsky Institute of Virology in Moscow. Subjects discussed were: (1) attempt at adaptation of leukemia viruses transplanted on Macaca arcotides and Papio hamadryas to conditions of cultivation in vitro, identification and characterization of these viruses; (2) search for identification and characterization of oncogenic viruses among the viruses isolated in tissue cultures of man and animals; (3) study of the mechanism of viral carcinogenesis. The plan of work included exchange of various biomaterials and reagents; study of exchangeable biomaterials and joint information; investigations and exchange of materials in the field of cancer control by immunological means; exchange of specialists; joint participation at principal conference on given problems; joint publications; and exchange of scientific information.

The next meeting of the US-USSR delegations will be held in Bethesda in May, 1975. A report of this meeting will appear in the next annual report.

Under the Agreement, Dr. T.A. Bektemirov of the Ivanovsky Institute for Virology in Moscow made an official visit to the U.S. in October-November, 1974. During his visit he worked with NCI scientists at Meloy Laboratories (Springfield, Va. and Rockville, Md.) and visited the laboratories of Dr. Thomas August and Dr. Sol Spiegelman in New York. He also toured the facility at the Frederick Cancer Research Center, attended the annual meeting of the Virus Cancer Program at Hershey, Pa., and visited with several scientists in the Viral Oncology Area, NCI.

Also under the Agreement, Dr. Walter Nelson-Rees, of the University of California at Berkeley, made an official visit to the Soviet Union in November, 1974. During this time he visited the Institute of Experimental and Clinical Oncology; the Institute of Medical Genetics; the Institute of Viral Preparations; the Institute of Virology; and the Institute of Epidemiology (Gamaleya) in Moscow.

Franco-American Exchange Program. This program permits the exchange of U.S. and French scientists to work and study in laboratories of the respective countries for periods up to three months. This year the following scientists participated:

U.S.: Dr. Victor Zeve - College de France, Paris
Dr. Paul Levine - International Agency for Research on Cancer, Lyon

France: Dr. Christine Simmler - NCI, Bethesda, Md.
Dr. Gilbert Lenoir - Flow Laboratories, Rockville, Md.

Ninth Annual Joint Working Conference - Virus Cancer Program. The annual meeting of the Virus Cancer Program was held at Hershey, Pa., on November 10-13, 1974. The following topics were discussed: molecular biology of DNA viruses associated with tumors; virion and viral-associated antigens; experimental animal models for DNA tumor viruses; natural and acquired immunity to RNA tumor viruses; the interaction of viral and host genomes in cancer; evidence for a human breast cancer virus; and the significance of primate RNA viruses to human cancer. The official program of this meeting may be obtained from this Office upon request.

Pacific Coast Tumor Virus Group (PACTVIGR). The Pacific Coast Tumor Virus Group (PACTVIGR) met on April 1 and September 30, 1974 at the California State Department of Public Health (Berkeley, California) and the Scripps Clinic and Research Foundation (La Jolla, California), respectively.

A summary of the accomplishments of the April 1 meeting follows: The highlights of the 16th PACTVIGR meeting centered on the discovery of the xenotropic mouse viruses which appear to be universal. The evidence in mice, together with the rescue of a xenotropic cat type C virus (RD114), have provided a basis for expanded efforts to rescue an analogous virus(es) in the human. Thus the techniques worked out for inducing the xenotropic viruses and studies of their behavior in their natural host systems are applicable to the search for the putative human agent.

Dr. Jay Levy discussed his work in delineating the host range of the xenotropic mouse virus. The virus was found to grow in almost all cells except mouse. This information and these systems were being applied to human cultures for rescue of the putative xenotropic human virus. Dr. M. Lieber noted that several of the endogenous mouse viruses grew well in a rabbit cell line; he described his experience in this and other cell lines. The major virus from BALB 3T3 cells was the xenotropic virus. He had also tested a number of exogenous viruses and found that the plasma Rauscher virus was a mixture of Rauscher and xenotropic viruses. Dr. Stuart Aaronson described neutralization experiments in which he found that the xenotropic endogenous mouse viruses stimulated neutralizing antibodies resulting in a widespread immunity in the strains studied. Dr. John Stephenson described the immunology of the xenotropic viruses. Drs. Suraiya Rasheed and M. Lee Vernon described their efforts to grow human placenta lines for rescue of the "human" type C virus visualized in the electron microscope. To date they had not been successful in inducing complete virus, but did have evidence of reverse transcriptase.

Dr. Helene Smith developed a method of eliminating fibroblasts in human cell cultures thus favoring epithelial cells. Dr. M. Namba described his studies on transforming human cells with carcinogenic chemicals. In some cases the lines were maintained into high passage levels.

Dr. R. Duff discussed the possible role of Herpes simplex virus (HSV) in human cancer. Tumors in hamsters induced by hamster cells transformed by HSV induced neutralizing antibody for both herpes types I and II.

Dr. Girardi rescued the transplantation antigen gene in SV40-transformed mouse cells. He presented evidence that this gene is incorporated into the long arm of the C7 chromosome.

Dr. Murray Gardner described efforts to prevent naturally occurring paralysis and lymphomas in a strain of wild mice. Both diseases were found to be caused by an endogenous type C virus isolated from wild mice. Guinea pig and mouse antisera were found to produce high levels of neutralizing antibody and protection against paralysis. Dr. Robert Huebner closed with a discussion of a number of ongoing antiviral vaccine experiments and approaches in a variety of mouse strains and animal species.

A summary of the accomplishments of the September 30 meeting follows: The 17th PACTVIGR meeting reported major progress by a number of laboratories in establishing the role of the endogenous type C RNA viruses in the etiology of natural and chemically induced cancers, including the previously reported discovery of the xenotropic type C viruses. This new class of endogenous type C viruses, which did not infect or replicate in host cells, were most likely responsible for those naturally occurring tumors with no evidence of ecotropic or other endogenous virus expression. The finding of natural antibodies to the xenotropic viruses gave impetus to the possibility of immunological control of virus expression under normal conditions; thus the cancer event might be postulated to occur with loss of immunological control.

Several interesting approaches aimed at regulatory systems governing virus expression were presented, including the following:

Dr. Michael Hanna discussed autogenous immunity to endogenous RNA tumor viruses. His group was now able to demonstrate natural antibody to the virus-envelope antigens by a radioimmune precipitation assay. By inducing immunity to murine leukemia-induced cell surface antigens in a highly immunocompetent newborn mouse, they were able to prevent the cancers induced by murine leukemia viruses. Thus the cell surface antigens served as effective and safe "vaccines", a procedure which could be highly relevant as an approach to human cancer.

Dr. Raymond Tennant described studies on inhibition of mouse leukemia virus infection by Fv-1 allele cell extracts. He found that treatment of mice with extracts made of N-tropic cell extracts inhibited infection by B-tropic viruses, but had no effect on NB-tropic viruses. BALB/c embryo cells infected with B-tropic virus showed a maximum inhibition, from 50-80%. In attempting to define the mechanism of viral inhibition, it was determined that the inhibitor apparently had no effect on cell growth.

Dr. Robert Huebner discussed vaccine studies against xenotropic murine leukemia viruses. His group had found that all factors were important: age, genetic susceptibility and resistance, etc. He pointed out that even in conventional virus infections, whether they produced disease depended on host responsiveness as well as the virus titer. Vaccine experiments currently underway included the following approaches: (1) administering vaccine to the mother, followed by boosters to the offspring; (2) testing the vaccines

in a number of genetic strains. Although it was too soon for definitive results, it appeared that the natural virus event, including cancer, could be interrupted by a vaccine made against the proper virus.

Dr. Murray Gardner found that wild mice from colonies with little antigen or virus expression had little and late cancer; and mice with high virus and viral expression had a high incidence of lymphomas and paralysis. There was a high correlation between virus titer and cancer. The incidence and age at onset of cancer was directly related to the virus titer.

Other papers dealt with some important new techniques for structural and biochemical definition of the type C viruses, as follows: Dr. Roy-Burman discussed phosphorylation of the different proteins in the assembly or replication of type C mammalian viruses. Dr. James Benson described a new technique for observing the structure of the inter- and intraspecies antigens of RNA tumor viruses. Dr. Norman Davidson had determined with the use of his technique of studying RNA molecules in the electron microscope that the RD114 virus had more genetic information than did the "standard" RNA tumor viruses. Dr. Margery Nicolson discussed transfection of mammalian cells with DNA from human cells productively infected with transformation-defective type C viruses. Human RD cells productively infected with RD114 DNA contained viral-specific integrated DNA capable of producing fully infectious virus identical to the parent virus. Dr. Gary Gerard described a highly sophisticated refinement of the reverse transcriptase procedure which consistently distinguished normal from cancer tissue. Ten of 19 cancer specimens were positive for reverse transcriptase; all of the control normal and fibroblastic tissues were negative. Breast cancer tissue appeared to have an uncommonly high ratio of positives. This group is now looking at human leukemic and human placenta cells.

Atlantic Coast Tumor Virus Group (ACTVIGR). The Atlantic Coast Tumor Virus Group (ACTVIGR) met only once during this fiscal year in deference to travel restrictions. The meeting was held at the Oak Ridge National Laboratory, Oak Ridge, Tennessee on December 9, 1974. About 50 participants gathered to discuss the topic, "Natural and Acquired Immunity to RNA Tumor Viruses". Under the Chairmanship of Dr. Fred Rapp the scientists presented information in the following areas: natural antibody to endogenous type C viruses; virus-induced cell surface components; interspecies reactivity among type C viruses; and prospects for vaccine modification of tumor incidences.

International Workshop on Production, Concentration, and Purification of Epstein-Barr Virus and Viral Antigens. This meeting, held February 10-12, 1974 at the NCI Frederick Cancer Research Center in Frederick, Maryland, was the first joint workshop of the National Cancer Institute and the International Agency for Research on Cancer which was proposed through the Reference Program for Oncogenic Herpesviruses of the IARC in December, 1973.

Six sessions dealt with the selection of cell cultures for virus production, the methods currently used for large scale EBV propagation, the methodology employed in obtaining EBV DNA and its use in nucleic acid hybridization experiments, and an exhaustive discussion of the various antigenic species induced by EBV in the various producer and nonproducer lymphoblastoid cell lines.

Among the specific recommendations made at the close of each session, those pertaining to the selection of cell cultures and the nature of the resulting EBV progeny were most useful. The participants at the workshop felt that the establishment of an information center or perhaps a reference laboratory function to authenticate the nature of a given cell line was very important. Markers such as HL-A type, sex chromosome, and isoenzyme pattern were suggested. Regarding the nature of the EBV produced, it was felt that the use of the terms "transforming" and "non-transforming" were in order rather than the previously used term "infectious". Some interest was shown in a multi-laboratory effort to characterize various lots of "transforming" EBV by the different tests now available, for example, cord blood transformation, EBNA induction, and stimulation of DNA synthesis. It was also felt that greater standardization of the biological assays used in EBV research should have a high priority.

Highlights of some of the material discussed in the session on nucleic acid research included the presentation of evidence for the detection of the EBV genome as a circular macromolecule in the Raji cell nucleus. Data from various laboratories supported the notion that the EBV genome may be carried in both an integrated and episomal state. A report of the first positive experiments resulting in the induction of malignancy in primates by a free nucleic acid molecule was made. Whether the Herpesvirus saimiri DNA was active, as such, in oncogeneis or was first active as an infectious DNA resulting in oncogenic virions could not be determined. Serologic follow-up of exposed animals was recommended.

The possibility of studying EBV antigens by exposing synchronized lymphoblastoid cells to pyrimidine analogues or inhibitors of protein synthesis was developed at some length. Two cytotoxicity tests, one applicable for Herpesvirus saimiri, the other for EBV "membrane antigen(s)", were described. In the latter case, evidence was presented which indicated that "membrane antigen" not detectable by the conventional immunofluorescence tests was present in nonproducer cells and was demonstrated by a T-cell cytotoxicity assay. Advances were reported relating to the isolation of the EBV-induced "early antigen(s)" and "nuclear antigen", yet while various laboratories can prepare specific proteins from antigen-bearing cells, little or no evidence relating to specific biologic function was presented. The general characteristics and isolation of "soluble antigen" complex was also outlined. The use of hydroxylapatite columns in studying these soluble antigen complexes was found to be most suitable for retention of antigenicity during purification.

Laboratory demonstrations of specific EBV research procedures were also carried out during the workshop. Such activities as hybrid cell formation and characterization, an iodine-labeled antibody assay for specific EBV antigens, the detection of the nuclear antigen and comments regarding the hazard of nonspecific immunodiffusion lines were presented. An evaluation of EBV produced in five different laboratories was likewise reviewed although few specific comments relating to how virus production could be improved were offered.

The proceedings will be published through the IARC as a booklet.

Symposium on the Characterization of Oncorna and Related Viruses. A symposium on the "Characterization of Oncorna and Related Viruses" was held September 22-24, 1974 at the Shoreham American Hotel, Washington, D.C. Separate sessions were held on the morphology, biochemistry, genetics, and immunology of RNA tumor viruses. The final session was concerned with a discussion of recommendations for the classification of these and related viruses. Ninety scientists from the United States and six foreign countries participated. In the session on morphology a historical review of the development of the concept of the oncornavirus group was presented. Information pointing to the presence of oncornaviruses in many animal species including man was discussed. The presence of type B viruses and the absence of type C virus in guinea pigs was documented.

In the biochemistry session ultrastructural characteristics of single stranded viral RNA were correlated with biochemical analysis. Other presentations involved discussions of intracellular virus-specific RNA, biological function of reverse transcriptase, and the evolution of type C viral information in relation to human leukemia. In the genetics session discussions evolved around interactions of endogenous mammary tumor viruses with their hosts, host gene control of avian oncornaviruses, and characterization of endogenous primate type C viruses. In immunology, virus-induced cell surface antigens, evolution and transmission of mammalian virogenes, immunologic markers of RNA tumor viruses, immunochemical and biochemical characterization of reverse transcriptase-containing viruses, and type B virus in baboons were discussed.

The final session was concerned with the development of recommendation on the classification of oncornaviruses. It was suggested that the term "Retraviridae" be given to the family of viruses containing RDDP and that the term "oncornavirus" be reserved for the genus of RNA viruses having the same ultrastructural, biochemical and immunological characteristics, whether or not individual viruses induced malignancy. Related viruses were: genus candidate oncornavirus D (Mason-Pfizer Monkey Virus), Genus Lentivirus E (visna-maedi viruses) and Genus Spumavirus F (the foamy viruses).

Summaries of this discussion and recommendations are to be published in Intervirology and the Journal of the National Cancer Institute.

Conference on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia. The Conference was held at the National Institutes of Health in Bethesda, Maryland, December 9-11, 1974, and was attended by over 130 investigators representing the United States and seven foreign countries. The objective of this Conference was to assess whether modulation of host immunity, effected through the use of defined chemicals or biologicals, would enhance the prevention or treatment of induced neoplasia.

The reports presented by several national and international investigators suggested that several chemicals (e.g., Levamisole, polyinosinic-polycytidylic acid, tilorone, and pyran copolymer) and interferon are excellent candidates for clinical trial. Several clinical reports indicated Levamisole was useful in decreasing the severity of recurrent Herpes labialis, apthous stomatitis,

warts, Crohn's disease, and rheumatoid arthritis. The protective effects were attributed to the immuno-stimulatory activity of Levamisole. Strong supportive evidence was presented indicating the usefulness of Levamisole as an adjuvant in combined treatment modalities for control of different tumors. Tilorone was reported to decrease or prevent further enlargement of bronchiogenic tumors. Interferon was reported to be effective in the treatment of Herpes zoster infections and of osteogenic sarcoma.

Reports presented at this Conference stimulated participants to apply this information to their basic and clinical studies. As a result, there are four clinical trials underway for using Levamisole in combined treatment protocols for the control of adenocarcinomas and leukemias and a Planning Conference has been established to implement a program for the production, testing, and use of interferon in treating osteogenic sarcomas and/or other tumors.

Symposium on Immunologic Control of Virus-Associated Tumors in Man: Prospects and Problems. The Immunology-Epidemiology Segment planned and implemented this symposium on behalf of the Virus Cancer Program in collaboration with the Fogarty International Center. The meeting was held on April 7-9, 1975 at the National Institutes of Health.

The symposium focused on four types of human tumors. Two of them, lymphoma and cervical cancer, are associated with well-characterized human viruses which have demonstrated oncogenic potential; namely, the Epstein-Barr virus (EBV) and Herpes simplex type 2, respectively. In the other two, leukemia and breast cancer, there is increasing evidence for a viral etiology, based primarily on experimental animal models. Epidemiologic, immunologic, and virologic aspects of avian, murine, feline and simian tumors related to these four types of human cancer were discussed with attention directed to their prevention and cure.

The importance of controlling oncogenic viruses as a means of preventing occurrence, reinduction, or relapse of disease was stressed, as was the need for considering any known antiviral measures in patients with diagnosed cancer. A variety of approaches were considered, including augmentation of immunity to viral and non-viral-associated tumor antigens, direct antiviral drugs, interferon stimulators, and transfer factor.

VIRUS CANCER PROGRAM

BIBLIOGRAPHY

July 1, 1974 - March 1975

Part A: Published Papers

Part B: Papers in Press

AUTHOR INDEX

Aaronson, S.A. / 1, 2, 3, 4, 35, 244, 245, 246, 247, 364, 617, 618, 619, 620, 621, 660, 661, 721, 722, 835, 1022
Aaslestad, D.R. / 811
Aaslestad, H.G. / 723, 1027
Ablashi, D.V. / 5, 6, 7, 8, 9, 10, 11, 147, 796, 800, 811, 1027
Abramson, J.H. / 12
Abtin, A. / 972
Adam, E. / 421, 525, 578, 579, 734
Adams, A. / 724, 725
Ahmed, M. / 13, 14, 15, 80, 563, 564, 716, 717
Aisenberg, A.C. / 16, 374, 726, 727, 728
Albert, S. / 385
Albrecht, T. / 17, 933
Aldenderfer, D.H. / 136
Alena, B. / 219
Alessi, A. / 564
Alevy, M. / 257
Alex, S.A. / 80, 81
Alford, C.A. / 448, 936
Ali, M. / 47
Allen, P.T. / 18, 19, 20, 88, 157, 223, 729, 730
Al-Sarraf, M. / 339
Altenburg, B. / 731
Ambrose, K.R. / 116, 117, 731, 842
Anderson, B. / 694
Anderson, N.G. / 21, 117, 118, 119, 261
Anderson, P.N. / 22, 23, 210, 340, 550
Anderson, R. / 733
Andersson, B. / 356, 893
Andre-Schwartz, J. / 489
Anglim, M.A. / 915
Anisowicz, A. / 864
Anver, M. / 133
Anzai, T. / 734
Aoki, T. / 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 112, 246, 284, 301, 350, 491, 735, 990
Arens, M. / 243, 582, 1048
Aristizabal, N. / 932
Armstrong, G.R. / 6, 7, 8, 11, 723, 800, 811
Arnold, J. / 36, 736, 737
Arnstein, P. / 37, 618
Arthur, L.O. / 38, 191
Asher, Y. / 750, 751
Ashley, R. / 1051
August, J.T. / 39, 73, 253, 310, 596, 597, 612, 625, 626, 627, 628, 629, 718, 738, 839, 1006
Aupoix, M. / 40, 740
Auld. D.S. / 739
Aurelian, L. / 41, 42, 43, 44, 335, 741, 742
Auriol, L. / 179
Axel, R. / 606, 608, 998

Baldwin, C. / 743
Ball, F.L. / 21
Ball, G. / 181
Baltimore, D. / 45, 70, 197, 408, 475, 679, 680, 892, 919, 920
Baluda, M.A. / 46, 47, 185, 186, 190, 590, 591, 807
Bandyopadhyay, A.K. / 48, 49, 50, 90, 136, 589
Barbeito, M.S. / 743
Barahona, H.H. / 51, 133, 744, 745
Barker, S.T. / 267
Barlati, S. / 746
Barnett, J.W. / 184
Baron, S. / 747
Barrera, O. / 301
Bassin, R.H. / 230
Bast, R.C. / 169
Battista, A. / 642, 643, 1013, 1014
Batzing, B.L. / 260
Baxt, W. / 606, 607, 998
Bazilier, M. / 177, 178, 358, 538, 812
Beagle, R. / 778
Beard, D. / 357, 748
Beard, J.W. / 357, 748
Becker, L.E. / 749
Becker, Y. / 52, 69, 348, 750, 751, 752, 753, 942
Beemon, K. / 54, 164, 165, 166, 808
Bekesi, J.G. / 55, 56, 57, 58, 59, 298, 299, 754, 755, 756
Belamaric, J. / 106, 339
Bell, C. / 604
Bellevue, R. / 757
Benacerraf, B. / 162

Ben-Bassat, H. / 60
Benedict, W.F. / 444, 890
Benirschke, K. / 61, 460
Benjamin, T. / 62, 592
Bentvelzen, P. / 63
Benveniste, R.E. / 64, 65, 66, 67, 103, 586, 658, 659, 758, 907
Benyesh-Melnick, M. / 68, 127, 128, 440, 560, 734, 884
Ben Zeev, A. / 53, 69, 751
Berard, C.W. / 901
Bernstein, A.D. / 120
Bernstein, I.D. / 700, 701
Besmer, P. / 70
Betuel, H. / 759
Bhaduri, S. / 242, 243, 582, 678
Bhattacharyya, J. / 71, 210
Bias, W.B. / 22, 71, 557
Biberfeld, P. / 430
Bigner, D.D. / 239
Bilello, J.A. / 72
Billing, R. / 74, 75
Biquard, J.M. / 40, 76
Bishop, J.M. / 77, 78, 126, 611, 647, 674, 675, 676, 677, 837, 838, 969, 1015
Biswal, N. / 440
Black, M.M. / 79, 670, 671, 760
Black, P.H. / 296, 297, 488, 489, 501, 859, 860, 861, 960, 961
Blaineau, C. / 761
Blankevoort, R. / 681
Blattner, W.A. / 877, 878
Blevins, C.S. / 192
Bloomer, M.J. / 764
Bloomfield, C.D. / 206, 207
Boaz, J. / 502
Boehm-Truitt, M. / 565, 566
Boettiger, D. / 145
Bogart, M.H. / 61
Bogden, A.E. / 80, 81, 183, 353, 354
Boiron, M. / 178, 359, 538, 645, 812, 894
Bolognesi, D.P. / 82, 83, 84, 239, 256, 307, 383, 384, 559, 762, 763, 840, 853, 870, 905
Bone, D.R. / 85
Bonkowsky, H.L. / 86, 764
Bont, W. / 291, 292, 858
Boone, C.W. / 92, 93, 228, 467, 768, 769
Booth, G.J. / 879
Borsos, T. / 169
Bouillant, A.M.P. / 765
Bourne, H.R. / 784, 785
Bowen, J.M. / 19, 20, 87, 88, 89, 107, 155, 156, 157, 158, 160, 161, 729, 730, 805
Bowling, J. / 136
Bowling, M. / 90
Boyd, A.L. / 422, 467, 768, 769
Boyse, E.A. / 310
Brack, M. / 91
Brackmann, K. / 243
Bradley, R. / 36
Brandchaft, P.B. / 92, 93
Braslawsky, G. / 336, 337
Breillatt, J.P. / 21
Brennan, M. / 385, 536
Briggs, R. / 96
Brown, A. / 569, 651, 713
Brown, B.L. / 441
Brown, B.O. / 94
Brown, J.C. / 215
Brown, T.D.K. / 770, 771, 814
Brunschwig, J.P. / 560
Buckley, P.M. / 95, 328, 329, 330
Buckner, C.D. / 96, 97, 98, 235, 314, 623, 624, 1003, 1004, 1017, 1018
Buehring, G.C. / 99
Buell, D.N. / 172, 542
Buell, P. / 772
Buettner, W. / 100, 243
Burke, P.J. / 22
Buss, E.R. / 516
Butel, J.S. / 422, 999
Buys, F. / 101
Byers, M.J. / 313
Byers, V.C. / 254, 609, 773, 901

Cachard, A. / 40
Cadwallader, J. / 420, 924
Calafat, J. / 101, 102, 291, 292, 529, 774, 857
Caldwell, G.G. / 775, 845
Calhoun, L. / 409
Callahan, R. / 64, 103, 658, 758, 776
Calloway, A. / 630
Calothy, G. / 483
Cameron, K.R. / 777
Cammoun, N. / 759
Campbell, A.K. / 113
Campbell, W.F. / 819
Campos, L.T. / 599, 600, 601, 1040
Canaani, E. / 104
Candler, E.L. / 21, 116
Cardiff, R.D. / 105, 652, 1051
Carpenter, C.B. / 488, 489
Carrico, R.J. / 778

Carrier, S.K. / 654, 655, 1019
Carta, M.A. / 636
Casagrande, J.T. / 277, 821, 954
Cassell, D. / 241
Cate, C.C. / 81
Caton, J.E. / 21
Cejka, J. / 106, 322, 339
Cervenka, J. / 733, 1029
Cetta, A. / 702, 1047
Chan, J.C. / 19, 88, 107, 171, 295
Chan, S.H. / 595, 779, 780, 781, 991
Chang, K.S.S. / 284
Chang, S. / 258
Chang, S.S. / 27
Chapman, W.H. / 687
Char, D. / 285, 922
Charman, H.P. / 108, 219, 823
Charney, J. / 109
Chattopadhyay, S.K. / 110, 111, 380
Chau, J.C.W. / 285
Chen, H.W. / 923
Chen, P.K. / 782
Chen, P.L. / 783
Chen, Y. / 204, 205, 820
Cherry, M. / 923
Chesner, J. / 426
Chew, T.S. / 779, 780, 781
Chieco-Bianchi, L. / 28, 112
Chirigos, M.A. / 496, 558, 951, 959
Cho, B.R. / 901
Cho, H.Y. / 531
Chretien, P.B. / 300
Christie, D. / 895
Chu, E. / 800
Cicmanec, J.L. / 15, 113, 114, 115, 506, 507, 688
Clark, R.L. / 598
Clawson, C.C. / 782
Cleton, F.J. / 857
Clifford, P. / 252
Clift, R.A. / 96, 97, 98, 235, 314, 623, 624, 653, 1003, 1004, 1017, 1018
Cobb, W.R. / 80, 81, 353
Cocchiara, R. / 643, 1013
Coccia, P.F. / 733
Cody, C.M. / 109
Coffino, P. / 784, 785, 766, 767
Coggin, J.H., Jr. / 21, 116, 117, 118, 119, 198, 261, 732, 842
Cohen, G.H. / 964
Cohen, J. / 786
Cohen, M.H. / 120, 121, 922
Cohen, Y. / 752, 753
Colcher, D. / 122, 123, 124, 125, 565, 566, 567, 714, 787, 984
Collavo, D. / 112
Collins, F.C. / 491
Colombatti, A. / 112
Comer, J. / 136
Comstock, J.P. / 973
Connelly, R.R. / 901, 904
Conway de Macario, E. / 386, 387
Cooper, R.W. / 187
Copeland, T. / 944
Cordell-Stewart, B. / 126, 1015
Cornish, J.D. / 742
Cottler-Fox, M. / 169
Courtney, R.J. / 68, 85, 127, 128, 734
Coutinho, W.G. / 362
Crawford, J. / 654
Croker, B.P. / 131, 143, 144, 154, 367, 802
Crow, J.D. / 619
Crusius, B. / 291
Culbreth, K. / 384

Dajbog, T. / 336, 337
Dalton, A. / 788
Daly, T.M. / 585
Dawson, E. / 915
Daniel, M.D. / 132, 133, 419, 789, 924
Daniel, R.W. / 965
Das, M.R. / 536
Davidson, N. / 351, 352
Davis, J. / 134, 790, 962
Day, N.E. / 595, 695, 759, 796, 797, 991
Dean, A.D. / 135
Dean, A.G. / 854
Dean, J.H. / 412, 922
Decker, B. / 231, 826
Decleve, A. / 289, 872
Deepak, J. / 90, 136
Dienhardt, F.W. / 137, 138, 139, 140, 141, 142, 187, 188, 368, 369, 401, 402, 403, 448, 562, 632, 633, 634, 697, 698, 778, 791, 792, 793, 794, 815, 899, 936, 982, 983, 1041, 1042,
Deinhardt, J.B. / 793
Delarea, J. / 336, 337
Del Guidice, R. / 844
Delius, H. / 390, 391
Del Villano, B.C. / 131, 143, 144, 154, 367, 802
Demoise, C. / 693
Deng, C.T. / 145, 674, 675
De Noronha, F. / 559
De Nucci, C. / 643
DePaoli, A. / 95, 328, 329, 330
Derge, J.G. / 258, 259, 453, 841
Desai, L.S. / 146

Desgranges, C. / 1049
DeSostoa, A. / 411
De Thé, G. / 7, 147, 148, 149, 437, 595, 696, 759, 781, 795, 796, 797, 798, 799, 811, 854, 932, 947, 991, 1049
DeVita, V.T. / 901
Diamond, R.D. / 370, 902
Didier, M.L. / 800
Dierlam, P.J. / 21, 117
Di Gioia, M. / 643, 1013
Di Mayorca, G. / 389, 699, 1043
Dion, A.S. / 150, 151, 152, 153, 670, 671
Disalvo, L.H. / 801
Diwan, B.A. / 817, 923
Dixon, F.J. / 131, 143, 144, 154, 367, 802
Dmochowski, L. / 18, 19, 88, 89, 107, 155, 156, 157, 158, 159, 160, 161, 171, 173, 174, 184, 223, 295, 395, 396, 397, 398, 399, 426, 497, 498, 499, 574, 575, 576, 577, 729, 730, 803, 804, 805, 916
Dobbs, J. / 136
Dodson, M.L. / 156, 160, 161
Doherty, J.M. / 86
Domenik, C. / 675
Donahoe, R.M. / 953
Dorf, M. / 162
Dorfman, R.F. / 901
Dorizzi, M. / 163
Dosik, H. / 388, 656, 757, 806, 1020
Dougherty, E. / 454
Doxy, A. / 646
Draper, G.J. / 366
Dreesman, G.R. / 734, 999
Drohan, W. N. / 46, 190, 807
Drost, S.D. / 680
Du Bose, D. / 789
Duesberg, P. / 54, 104, 164, 165, 166, 390, 391, 684, 689
Duff, R. / 167, 168, 345, 517, 518, 579, 809, 810, 934, 963
Dunkel, V.C. / 169
Dunn, K.J. / 449
Duran-Reynals, M.L. / 372, 910
Dux, A. / 170, 436

East, J.L. / 19, 20, 88, 107, 157, 171, 397, 498, 729, 730
Easton, J.M. / 6, 7, 811, 901
Ebert, P.S. / 86, 172, 764
Eckner, R.J. / 173, 174
Eichberg, J. / 91, 175, 876
Elquezabel, A. / 1020
Elkins, W.L. / 176
Eller, J.J. / 346, 876
Ellis, D.A. / 297, 861
Ellouz, R. / 147, 759
Emanoil-Ravicovitch, R. / 177, 178, 179, 358, 538, 812
Emmelot, P. / 292, 858, 1028
Epstein, A.L. / 180
Epstein, M.A. / 181
Epstein, R.B. / 622
Ernberg, I. / 182, 594, 770, 813, 814
Esber, H.J. / 81, 183, 353, 354
Esparza, J. / 68, 884
Essex, M. / 404
Estes, J.D. / 217, 218, 276, 821, 972
Estes, M.K. / 999
Evans, D.L. / 184
Evans, R.M. / 46, 47, 185, 186, 590
Eveleigh, J.W. / 21

Fabrizio, P. / 904
Fagraeus, A. / 220
Falk, L. / 141, 142, 187, 188, 401, 448, 697, 698, 794, 815, 936, 982, 1041, 1042
Fan, H. / 70
Fan, W. / 676
Farrelly, J.G. / 309, 648, 649
Farrugia, R. / 232
Favre, M.Cl. 147, 1049
Feeney, M. / 604
Fefer, A. / 96, 97, 98, 235, 314, 623, 624, 653, 1003, 1004, 1017, 1018
Feldman, S.P. / 567, 926, 984
Felix, E. / 922
Felsburg, P.J. / 876
Feltkamp, C. / 292
Fenyo, E.M. / 189
Feorino, P.C. / 845
Fialkow, P.J. / 886
Fidanian, H.M. / 190
Fields, B.N. / 603, 631
Fine, D.L. / 191
Fink, M.A. / 121
Firtel, H.F. / 679
Fischer, R.G. / 966
Fischinger, P.J. / 112, 192, 193, 239, 449, 450
Fish, D.C. / 136
Flandermeyer, R.R. / 446
Flannery, V.L. / 885

Fleisher, T. / 194, 195
Floersheim, G.L. / 196, 347
Foreman, C. / 469
Forget, B.G. / 197
Fortner, G.W. / 198
Fout, G.S. / 150, 151, 152, 153
Fowler, A.K. / 269, 320, 816, 848
Fox, R.R. / 817
Fraenkel-Conrat, H. / 255
Frankel, J.W. / 818, 819
Fraser, C.E.O. / 133, 199, 200, 419, 924
Fraumeni, J.F. / 877, 878
Freeman, A.E. / 664, 869
Freireich, E.J. / 286
Friedman, M. / 201
Friis, R.R. / 204, 205, 257, 306, 400, 686, 820, 840
Fudenberg, H.H. / 773, 900
Fujinaga, K. / 202, 203, 243
Fujita, D. / 204, 205
Funk, D.D. / 96, 97
Funk, F. / 17
Furmanski, P. / 413, 414, 536, 545, 974

Gajl-Peczalska, K. / 206, 207, 208, 333, 880
Galesloot, J. / 289
Gallagher, R.E. / 71, 209, 211, 212, 213, 214, 427
Gallo, R. / 71, 209, 210, 211, 212, 213, 214, 406, 427, 477, 500, 527, 528, 550, 551, 555, 556, 580, 657, 702, 703, 704, 705, 706, 707, 708, 709, 967
Ganesan, J. / 646
Garcia, F.G. / 51, 419, 420, 744
Gardner, M.B. / 215, 216, 217, 218, 219, 276, 540, 541, 554, 821, 918, 972
Garfin, D.E. / 647
Gatti, R.A. / 220, 632, 715
Gazdar, A.F. / 221, 222, 284, 552, 747, 952
Gehrtz, R. / 195
Geissler, E. / 315
Gentry, W. / 1029
Georgiades, J. / 18, 19, 20, 88, 157, 223, 729, 730
Gerard, G. / 224, 240, 241, 242, 822
Gerkins, V.R. / 850
Gerwin, B.I. / 169, 230
Geser, A. / 148, 695, 797, 798
Gheridian, B. / 960, 961
Gianni, A.M. / 678
Giard, D.J. / 878
Gibson, W. / 225
Gilden, R.V. / 108, 219, 226, 269, 276, 379, 459, 463, 468, 469, 508, 532, 534, 663, 664, 667, 668, 790, 823, 911, 918, 939, 944, 945, 962
Gillespie, D. / 211, 213, 214, 427
Gillette, R.W. / 227, 228, 229, 824
Giovanella, B.C. / 886
Gisselbrecht, S. / 230
Girardi, A.J. / 129, 130, 539
Glancy, T. / 413, 415
Glaser, R. / 231, 232, 233, 234, 345, 417, 418, 825, 826, 827, 828, 829, 830, 831, 832
Gleichmann, H. / 488
Gloub, S.H. / 632, 633
Gluckman, E. / 623, 986
Gluckman, J.C. / 700
Glucksberg, H. / 96, 97, 235, 623, 624, 1004, 1018
Goepfert, H. / 578
Goldberg, R.J. / 236, 571
Goldblum, N. / 60
Goldman, E. / 62
Goltz, R. / 381, 1029
Gomex, M. / 778
Gonda, M.A. / 720
Good, R.A. / 334, 715
Goodheart, C.R. / 8, 237, 429, 493, 494, 823
Goodman, H. / 126, 647, 1015
Gordon, R.G. / 851
Gordon, R.J. / 955
Goselink, H. / 831, 988
Graf, T. / 83
Graham, T.C. / 347, 455, 622, 623, 692, 831, 987, 1005, 1023, 1036, 1037
Grandgenett, D. / 238, 241, 242, 822, 834
Grant, J. / 84, 239
Grant, P.M. / 413, 414, 415, 416
Gravell, M. / 371, 903
Gray, J.W. / 785
Green, M. / 110, 202, 203, 224, 238, 240, 241, 242, 243, 375, 376, 535, 582, 665, 678, 710, 971, 1048
Green, R.W. / 83, 559, 853
Green, W. / 564
Greenberger, J.S. / 4, 244, 245, 246, 247, 835
Greig, A.S. / 765
Gries, C. / 454
Groff, D.E. / 313

Grose, C. / 836
Groupé, V. / 819
Gschwind, C.R. / 286, 287, 288
Guard, H.E. / 801
Guerra, A. / 248
Guirgis, H.A. / 385
Guntaka, R.V. / 674, 675, 676, 837, 838
Gunven, P. / 249, 250, 251, 252, 632, 633
Gupta, P. / 253, 839
Gurgo, C. / 241
Gussoff, B.D. / 757
Gutterman, J.U. / 286, 287, 288
Györkey, F. / 992, 995, 1033

Haas, M. / 872
Haberman, A.B. / 304
Hackett, A.J. / 99, 254, 609, 610, 773, 900
Hadidi, A. / 456, 457, 458, 938
Hageman, Ph.C. / 101, 102, 292, 529, 774, 857
Hales, R.L. / 158, 295
Hallgren, H. / 208, 333, 880
Halpern, M.S. / 256, 257, 820, 840, 905
Hamelin, R. / 359, 462, 645, 894
Hamilton, B. / 700
Hampar, B. / 258, 259, 453, 841
Hampe, A. / 358
Hanafusa, H. / 29
Hanisch, R. / 277
Hanna, M.G., Jr. / 198, 260, 287, 288, 307, 364, 648, 649
Hannon, W.H. / 116, 261, 842
Hanson, M. / 823
Hards, E.J. / 268
Hardy, W.D., Jr. / 303
Hargreaves, R.E. / 701
Harris, P. / 685
Harris, R. / 604
Harrison, T.A. / 408, 919, 920
Hartley, J.W. / 449, 843, 958
Harvey, J.S., Jr. / 876
Haseltine, W. / 70
Haspel, M.V. / 262, 263, 518
Haspel, O. / 348
Hatanaka, M. / 379, 459, 663, 664, 666, 667, 668, 844, 911, 939
Haverman, J. / 291, 292, 858, 859
Hawthorne, P.K. / 446
Hay, K.A. / 895, 896, 897
Hayflick, L. / 265, 435, 1044, 1045, 1046
Heasley, S. / 676, 677
Heath, C.W., Jr. / 509, 775, 845
Heberling, R.L. / 91, 266, 267, 319, 320, 321, 346, 846, 874, 875, 876, 996
Heding, L. / 941
Hehlmann, R. / 608
Heidel, J.W. / 847
Heine, U. / 9, 167
Heinemann, R. / 64
Heiniger, H.J. / 268, 923
Hekman, A. / 101
Helinski, D. / 363
Hellman, A. / 267, 269, 330, 816, 848, 874, 875
Hellstrom, I. / 176, 270, 271, 272, 273, 274, 639, 687, 701, 849, 873
Hellstrom, K.E. / 176, 270, 271, 272, 273, 274, 687, 700, 849, 935
Helmke, R.J. / 320, 321, 875, 996
Henderson, B.E. / 215, 216, 275, 276, 277, 772, 821, 850, 851, 862, 912, 925, 955, 956
Henderson, B.H. / 1034
Henle, G. / 229, 280, 281, 282, 283, 305, 827, 836, 852, 863, 871, 1008
Henle, W. / 248, 279, 280, 281, 282, 283, 305, 772, 827, 836, 852, 863, 871, 1008
Herberman, R.B. / 412, 904, 922
Herles, S. / 13
Herman, A.C. / 853
Hernandez-Camacho, J.I. / 187
Hersh, E.M. / 89, 286, 287, 288
Heubel, G.A. / 153
Hewetson, J.F. / 282, 711
Hieda, W. / 443
Higuchi, T. / 315
Hilgers, F. / 135, 355, 854, 855
Hilgers, J.H.M. / 101, 102, 289, 290, 291, 292, 529, 605, 673, 774, 856, 857, 858, 872, 997, 1028
Hilkmann, H.A.M. / 1028
Hill, P.R. / 553, 604, 979
Hilleman, M.R. / 293, 294
Hiraki, S. / 159
Hirsch, M.A. / 296, 297, 488, 489, 501, 859, 860, 861, 960, 961
Hirshaut, Y. / 451
Hisserich, J.C. / 862
Ho, J.C. / 285, 305
Ho, J.H.C. / 796, 797, 799
Ho, T. / 690, 712
Holben, J.A. / 109
Hoekstra, J. / 632, 633
Hokeness, S. / 881

Holder, H. / 284
Holladay, D.W. / 21
Holland, J.F. / 55, 56, 57, 58, 59, 298, 299, 755, 756
Holleman, J.W. / 21
Hollinshead, A.C. / 300
Hollis, V.W., Jr. / 28, 30, 301, 486
Holm, G. / 871
Hoover, E. / 464
Hopkins, K. / 557
Horecker, B.L. / 596, 597
Hoshino, M. / 158, 159
Horwitz, C.A. / 279, 283, 863
Howe, C.D. / 599, 601, 994
Howk, R.S. / 304, 864
Hsu, K.C. / 35
Huang, C.-H. / 451, 947
Huang, D.P. / 305
Huang, E.S. / 473, 542
Huang, R.C.C. / 611
Huebner, F.K. / 129, 130
Huebner, R.J. / 219, 276, 484, 485, 496, 531, 532, 533, 534, 618, 664, 821, 865, 959, 979
Hung, C.Y. / 866, 867
Hunsmann, G. / 307, 559
Hunt, R.D. / 51, 132, 133, 419, 420, 789
Hunter, E. / 257, 306
Hunter, L. / 801, 868
Hunter, S. / 575, 576, 577
Hüper, G. / 83, 307, 559, 763
Hutchison, D.J. / 783
Hutt, R. / 549

Igel, H.J. / 869
Ihle, J.N. / 260, 307, 308, 309, 364, 712
Ikeda, H. / 310
Ilyia, K.V. / 499
Ingalls, J.K. / 744
Isaacs, H. / 918
Ishimoto, A. / 311, 312, 443
Ishizaki, R. / 870
Ito, Y. / 311, 312, 442, 443, 763

Jackman, D. / 420, 924
Jain, D. / 553, 978, 979
Johansson, B. / 871
Johnson, H.M. / 747
Johnson, K. / 604
Johnson, R.W. / 589
Johnson, S. / 831, 988
Jones, C. / 895, 896, 897
Jongstra, J. / 872
Joseph, B.S. / 317

Kacian, D. / 714
Kahn, D. / 464
Kall, M.A. / 873
Kalter, S.S. / 91, 175, 266, 267, 269, 318, 319, 320, 321, 846, 874, 875, 876, 891, 996
Kamincik, Y. / 751
Kamlag, D. / 1028
Kan, Y.W. / 646
Kao, G.F. / 1036, 1037
Kaplan, H.S. / 180, 289
Kaplan, J. / 322, 323, 324, 325, 326, 327
Kaplan, M.B. / 541
Kaplan, M.M. / 877, 878
Karby, S. / 336, 337
Katz, E. / 686
Katz, M. / 167
Kawaguchi, H. / 739
Kawai, Y. / 451
Kawakami, T.G. / 95, 328, 329, 330, 661
Kay, H.D. / 331, 332, 598, 599, 600, 601, 654, 655, 992, 993, 994, 1019, 1033
Kelloff, G.J. / 484, 485, 953, 980
Kelly, A.P. / 859, 860
Kennel, S.T. / 367
Kenney, F.T. / 308, 309
Kersey, J. / 194, 195, 206, 207, 208, 333, 334, 381, 382, 445, 672, 733, 782, 879, 880, 881, 882, 883, 915, 1029
Kessler, I.I. / 335
Keydar, I. / 336, 337
Keys, C. / 277
Kiefer, R. / 349
Killander, D. / 871
Kim, E.B. / 532
Kim, N. / 108
Kimball, P. / 424, 565, 566, 570
Kimura, S. / 68, 884, 885
Kind, P.D. / 816
King, D. / 993
King, N.W. / 133
Kingsbury, D.T. / 338
Kinlen, L.J. / 602
Kinoshita, K. / 638
Kishida, T. / 761
Kithier, K. / 106, 339
Kiuchi, M. / 943
Klecker, J. / 604
Klein, D.L. / 22, 23, 340
Klein, E. / 189, 344, 356, 893, 1000

Klein, G. / 182, 252, 341, 342, 343, 344, 355, 423, 430, 594, 632, 633, 634, 715, 770, 771, 855, 871, 886, 887, 947, 1000
Klein, R.A. / 379
Kleinfeld, K.L. / 869
Knesek, J.E. / 171
Knight, P. / 345, 518
Kniker, W.T. / 175, 346, 876
Kociba, G.J. / 302, 303
Koh, C.K. / 712
Kohne, D. / 502, 503
Kolb, H.J. / 347, 622
Koldovsky, U. / 248
Koment, R.W. / 519, 888, 889
Koprowski, H. / 129, 130
Korol, W. / 13, 14, 15, 564
Kotler, M. / 348
Kouri, R.E. / 349, 444, 693, 890
Kourilsky, F.M. / 182, 594
Kouttab, N.M. / 816, 848
Kraemer, D.C. / 875, 891, 996
Kramarsky, B. / 783
Krakowska, S. / 465
Kramer, D.C. / 320
Kreider, J.W. / 167
Krivit, W. / 733, 881
Krontiris, T.G. / 603
Kruse, R. / 562, 983
Krycove, C. / 746
Kudo, T. / 28, 30, 350
Kufe, D. / 606, 607, 608, 998
Kung, H. / 351, 352
Kung, P.C. / 892
Kuntz, R.E. / 321
Kuo, E.Y.H. / 81, 353, 354
Kurtz, S. / 890
Kurylo-Borowska, Z. / 18, 223
Kvedar, J.P. / 30
Kwa, H. / 605, 681, 1032
Kwa, S.B. / 997

Ladda, R. / 231
Lai, M.M.C. / 164, 165, 166, 808
Lamie, E. / 290
Lamon, E. / 694, 855
Lamon, E.W. / 355, 356, 893
Lamzardi, G. / 564
Lane, J.A. / 496
Lane, S.E. / 309
Langlois, A.J. / 870
Lapis, K. / 357
Larsen, C.J. / 177, 358, 359, 360, 393, 894
Larson, D.L. / 716, 717
Larson, L. / 410
Lasfargues, E.Y. / 361, 362, 585, 969
Laug, W.E. / 918
Lausch, R.N. / 264, 438, 439, 495, 583, 895, 896, 897
Lavoue, M.F. / 797
Lavrin, D.H. / 284
Lawrence, F. / 970
Leavitt, R.W. / 363
Lebiedzik, J. / 720
Leblanc, D. / 1008
Lee, J.C. / 364
Lee, K.L. / 365
Lehner, T. / 898
Leibold, W. / 343, 423, 632, 887
Leighton, P. / 366
Leikola, J. / 282
Leis, H.P., Jr. / 79
Leis, J. / 84, 555
Lelek, J. / 615, 616
Lemp, J.F. / 743
Lennette, E.H. / 37, 537, 968
Lenoir, G. / 786
Lerner, K.G. / 235, 623, 1004, 1018, 1037
Lerner, R. / 131, 144, 154, 338, 363, 367, 502, 503, 802
Leventhal, B.G. / 285
Levin, A.C. / 368, 369, 402, 403, 899
Levin, A.S. / 111, 380, 773, 900
Levine, P.H. / 7, 120, 285, 286, 370, 371, 451, 811, 901, 902, 903, 904
Levinson, W.E. / 647
Levy, B. / 885
Levy, B.M. / 167
Levy, J.A. / 37
Lewandowski, L.J. / 256, 905
Li, J.N. / 520, 906
Lieber, M.M. / 103, 278, 658, 659, 758, 765, 776, 907, 908, 909
Lie-Injo, L.E. / 646
Lilly, F. / 162, 201, 372, 373, 627, 628, 910, 1038, 1039
Lindahl, T. / 343, 724, 725, 886
Ling, H.P. / 379, 911
Ling-Tsang, A. / 153
Linial, M. / 400
Links, J. / 101
Liu, M. / 31
Livingston, D.M. / 146, 739
Lodish, H.F. / 679
Loeb, W.F. / 114, 115, 506
Log, T. / 979
Loh, K.K. / 992, 993, 994
Long, C. / 508, 543, 666, 823, 844, 962

Long, J.C. / 16, 374, 727
Long, W.K. / 448, 936
Longley, C. / 414
Loni, M.C. / 242, 375, 376
Lopez, D.M. / 163, 377, 378
Louie, E. / 772
Lovinger, G.G. / 379, 911
Lowry, G. / 615, 616
Lowy, D.R. / 110, 111, 380
Lubiniecki, A.S. / 877, 878
Luckasen, J.R. / 195, 333, 381, 382
Luecke, D.H. / 966
Luftig, R.B. / 383, 384
Lukeman, M.J. / 577
Lund, J.K. / 105
Lyles, J.M. / 716, 717
Lynch, H.T. / 385

Macario, A.J.L. / 386, 387, 796
Macias, E.G. / 346, 876
Mack, T. / 925
MacPherson, I. / 145
Madahar, D.P. / 388
Madison, R. / 980
Magrath, I.T. / 912
Mahoney, M.J. / 764
Mahy, B. / 838
Major, E.O. / 389
Manaker, R.A. / 950
Mangel, W.F. / 390, 391
Manny, N. / 60
Manthey, W.J. / 977
Marcus, R.L. / 44
Markham, P.D. / 46, 47, 591
Marotta, C.A. / 197
Marshal, B.M. / 420
Martin, S. / 851
Martin, S.E. / 913, 914
Martin, S.P. / 862, 925
Martin, W.J. / 392, 913, 914
Martinson, I.M. / 915
Marty, M. / 359, 393, 894
Maruyama, K. / 19, 20, 88, 394, 395, 396, 397, 398, 399, 426, 729, 730, 916
Maryak, J.M. / 572
Mason, M.M. / 80
Mason, T. / 877
Mason, W.S. / 400, 680, 820, 840
Massey, R. / 142, 368, 369, 401, 402, 403, 899, 983
Mastenbroek, N. / 529
Mastrangelo, R. / 323
Mattern, C.F.T. / 449
Mathes, L.E. / 404, 465, 466, 941
Mauchauffé, M. / 177, 358, 393
Maurer, B.A. / 405
Mavligit, G. / 286, 287, 288
Mayer, R.J. / 211, 406
Mayyasi, S. / 13, 14, 716, 717, 1021
McAllister, R.M. / 351, 352, 407, 537, 540, 917, 918, 968
McBride, C.M. / 89
McCaffrey, R.P. / 197, 408, 892, 919, 920
McCaw, B.A. / 467
McClure, H.M. / 61, 115, 921
McCombs, R.M. / 560
McConahey, P.J. / 313, 367
McCormick, J.J. / 409, 410, 411, 536
McCoy, J.L. / 285, 405, 412, 904, 922
McCullough, B. / 175, 876
876
McGrath, C.M. / 411, 413, 414, 415, 416, 536, 546
McGrath, L.M. / 650
McKenzie, G.O. / 229
McKenzie, I.F.C. / 31, 960, 961
McMillon, P.N. / 383, 384
Medeiros, E. / 77
Meier, H. / 268, 817, 923
Mele, J. / 417, 418
Melendez, L.V. / 51, 132, 133, 199, 200, 419, 420, 744, 745, 789, 924
Mellors, A.J. / 521
Mellors, R.C. / 310, 718
Mellstedt, H. / 871
Melnick, J.L. / 421, 422, 525, 579, 614, 659, 745, 1001
Menck, H. / 772, 851, 925, 955
Menezes, J. / 343, 423, 887
Menninger, F.F., Jr. / 183
Merrill, J. / 488, 489
Meyers, P. / 378
Michalides, R. / 424, 482, 565, 566, 567, 913
Mickelson, E. / 455
Miller, J.M. / 823
Miller, N.F. / 19, 88, 89, 156, 158, 160, 161, 398, 425, 426, 729, 730
Miller, N.R. / 211, 214, 427
Milo, G.E. / 941
Minna, J.D. / 221, 222
Minowada, J. / 428

Mirand, E.A. / 173, 174
Mishra, N.K. / 978
Mitchen, J.R. / 819
Miyagi, M. / 237, 429, 493, 494, 833
Mizuno, F. / 946
Moenning, V. / 559
Molnar, Z. / 430
Moloney, W.C. / 247, 835
Monaco, A.P. / 861
Mondal, H. / 211
Montagnier, L. / 683
Moore, D.H. / 79, 109, 151, 152, 153, 361, 431, 432, 433, 434, 670, 671, 928, 929, 930, 931
Moore, G.E. / 428
Moore, G.T. / 891
Moorhead, P. / 435
Mourali, N. / 147
Mühlbock, O. / 170, 436
Muir, C. / 799
Mullin, B.C. / 690
Mullins, G.M. / 22
Munoz, N. / 437, 798, 932
Murasko, D.M. / 438, 439, 896, 897
Murray, B.K. / 440
Myer, F.E. / 650, 713
Myers, B. / 156, 729, 730

Nachtigal, M. / 933, 934
Nagle, S.C. / 94, 441
Nakagawa, T. / 1049
Nakajima, K. / 442, 443
Narayan, O. / 316, 749
Nash, M.A. / 729, 730
Nathenson, S. / 201
Nave, B. / 144
Nazerian, K. / 189
Neauport-Sautes, C. / 594
Nebert, D.W. / 444
Neely, A. / 445, 882
Neiman, P.E. / 96, 97, 98, 235, 623, 624, 653, 1003, 1004, 1018
Nelson, K. / 935
Nelson-Rees, W.A. / 61, 446, 918
Nesbit, M.E. / 206, 333, 733, 880, 881, 915
Neubauer, R.H. / 114, 447, 506, 507, 688
Neuburger, C. / 915
Neva, F.A. / 115
Newton, W.A. / 20, 729, 730
Nicholls, W.W. / 435
Nickol, J.M. / 365
Nicolson, M.A. / 351, 352
Nigida, S. / 187, 448, 936
Nilsson, K. / 343
Nims, R.M. / 980
Nishikawa, S. / 443
Nishioka, M. / 849
Nomura, S. / 192, 193, 449, 450
Nonoyama, M. / 233, 258, 259, 417, 418, 428, 451, 452, 453, 640, 827, 841, 937, 946, 1010
Noronha, F. / 454
Nunn, M. / 284
Nusse, R. / 102, 291, 292, 774, 857, 858

Ochs, H.D. / 347, 455, 623, 692
O'Connor, T.E. / 456, 457, 458, 938
O'Conor, G.T. / 901
Officer, J.E. / 217
Ogburn, C.A. / 1052
Ohno, T. / 714
Oie, H.K. / 800
Okabe, N. / 459, 939
Okano, P. / 456, 457, 458, 938
Oldham, R. / 285, 922
Olding, L.B. / 460
Oldstone, M.B.A. / 317, 460, 461, 940
Olofsson, B. / 462, 645
Olpin, J. / 463
Olsen, R.G. / 404, 464, 465, 466, 941
Olshevsky, U. / 53, 942
Olweny, C. / 912
Ono, K. / 442, 443
Opelz, G. / 943
Orme, T. / 467, 768, 769
Oroszlan, S. / 134, 269, 463, 468, 469, 790, 944, 945, 962
Orr, T. / 507
Ortiz-Muniz, G. / 163, 377
Osato, T. / 946
Oshiro, L.S. / 37
Ostborn, A. / 220
Otten, A. / 470
Owor, R. / 912

Paciga, J. / 401, 794
Pagano, J.S. / 428, 451, 471, 472, 473, 542, 947
Pal, B.K. / 540, 541, 948
Pang, R.H.L. / 487
Panigel, M. / 320, 874, 875, 996
Papas, T.S. / 558
Paran, M. / 477, 500, 706, 707, 708 709

Park, J.J. / 476, 486, 487
Parker, J. / 217, 218
Parkman, R. / 920
Parks, W.P. / 236, 304, 479, 481
571, 572, 719, 864, 949, 1050
Parr, M. / 455
Paskind, M. / 70
Paul, P.S. / 978
Paulsen, A. / 97
Pearson, G. / 10, 506, 507, 542
796, 932, 950
Peebles, P.T. / 269
Peer, M. / 918
Périés, J. / 179, 359, 761, 894
Perk, K. / 482, 951
Perkins, E.H. / 866, 867
Perkins, F.T. / 435
Perlin, E. / 922
Perry, A. / 589
Peters, R.L. / 952, 980
Peters, W.P. / 607
Peterson, C. / 499
Peterson, W.D., Jr. / 106, 322,
323, 324, 325, 326, 327, 339
Phillips, L.A. / 476, 486, 487
Pike, M.C. / 215, 366, 490, 602, 772,
821, 850, 851, 925, 954, 955,
956, 957
Pincus, T. / 310, 958
Plata, E.J. / 28, 30, 286, 491
Plowman, J.L. / 492
Plummer, G. / 493, 494
Poco, A. / 454
Poli, J. / 393
Pollack, S.B. / 935
Pomeroy, B.S. / 978
Portugal, F.H. / 669
Post, J. / 454
Potter, M. / 908
Poulik, M.D. / 339
Powell, D. / 277
Pretorius, G. / 831
Price, P. / 890
Price, P.J. / 37, 496, 554, 959
Priori, E.S. / 20, 88, 156, 158,
159, 160, 161, 173, 174, 497,
498, 499, 729, 730
Prival, J. / 500, 707, 708
Proffitt, M.R. / 297, 501, 859,
860, 861, 960, 961
Puentes, M.J. / 105
Puga, A. / 502, 503

Qazi, R. / 728
Quak, J. / 529
Quarles, J.M. / 470, 847
Quintrell, N. / 77

Rabin, H. / 5, 114, 181, 447, 505,
506, 507, 688, 950
Rabson, A. / 800, 932
Rabstein, L.S. / 484, 485, 980
Rand, K.H. / 508, 962
Rangan, S.R.S. / 581
Rankin, A. / 241
Ransom, J.C. / 480, 949
Rapp, F. / 17, 167, 168, 234, 262,
263, 345, 418, 511, 512
513, 514, 515, 516, 517,
518, 519, 520, 521, 522,
523, 524, 549, 579, 810
826, 828, 829, 830, 888, 889,
895, 906, 933, 934, 963,
964, 965, 976
Rasheed, S. / 219
Rauch, A.M. / 428
Rawls, W. / 300, 421, 525, 578, 579,
734, 999
Reed, C.L. / 522
Rehacek, J. / 966
Rein, A. / 230
Reisher, J.I. / 370, 902
Reisinger, R.C. / 526
Reissig, M. / 581, 965
Reitz, M.S. / 427, 527, 528, 551,
706, 709
Ressang, A.A. / 529
Rhim, J.S. / 530, 531, 532, 533, 534
Rho, H.M. / 242, 834
Rich, M.A. / 409, 410, 411, 413, 414,
415, 416, 536, 974
Richardson, L.S. / 477, 967
Rickard, C. / 454
Ricklis, S. / 534
Riggs, J.L. / 537, 968
Riggs, J.R. / 610
Ro, H.S. / 532, 533
Robert-Robin, J. / 177, 178, 179,
358, 812
Roberts, J.A. / 581
Robin, M.S. / 971
Robins, M. / 242
Robinson, O.R. / 589
Roboz, J.P. / 56, 57, 58, 59, 298,
299, 755, 756
Rocchi, G. / 282
Rogart, W. / 72
Rohde, W. / 126, 1015

Rohner, T.J. / 976
Roloff, J. / 881
Romero, J.J. / 332, 600, 654, 655, 992, 1019, 1040
Rommel, F.A. / 876
Rongey, R.W. / 217, 218, 219, 821, 918, 973
Rosario, I. / 277
Roseberry, E.A. / 528
Rosen, A. / 355, 855
Rosenthal, D.S. / 835
Roth, I. / 630
Rottman, F. / 224
Rowe, W.P. / 110, 111, 372, 380, 843, 910, 958
Roy-Burman, P. / 540, 541, 948
Rubio, V. / 543
Rudolph, R.H. / 455
Russell, E. / 221, 222, 952
Russo, J. / 536, 544, 736, 737

Sabad, A. / 195, 333, 381, 445, 672, 880, 882, 1029
Sabin, A.B. / 548, 644
Saby, J. / 544
Sachs, L. / 60
Sadowski, J.M. / 975
St. Jeor, J.C. / 17, 523, 549, 830
Salle, M. / 179, 761
Salsberg, S. / 242, 971
Sanford, E.J. / 524, 975
Santos, G.W. / 22, 23, 340
Sarin, P.S. / 550, 551
Sarkar, N.H. / 783, 977
Sarma, P.S. / 552, 553, 554, 978, 979
Sarngadharan, M.G. / 406, 555, 556, 702
Sass, B. / 980
Saxe, N. / 464
Saxinger, W.C. / 211, 214, 427
Scanlon, M. / 160, 161, 426
Schacter, B. / 557
Schafer, M.P. / 558
Schafer, W. / 84, 307, 559
Schaffer, P.A. / 68, 560, 884, 885, 981
Schaller, J.P. / 561, 941
Schauf, V. / 369, 474, 562, 899, 982, 983
Schenley, C.L. / 260
Schidlovsky, G. / 13, 14, 15, 563, 564
Schiop-Stansly, P. / 456, 457, 458, 938
Schlom, J. / 122, 123, 124, 125, 424, 482, 565, 566, 567, 568, 570, 606, 607, 608, 714, 721, 787, 926, 927, 984, 985, 998
Schluter, B. / 569, 651, 713
Schmitz, H. / 863
Schochetman, G. / 122, 565, 566, 570, 984, 985
Schroeder, M.L. / 692, 831, 986, 987, 988, 1005, 1023, 1024
Schwartz, R.S. / 488, 489
Scolnick, E.M. / 236, 304, 479, 480, 489, 571, 572, 719, 864, 949, 1050
Sekikawa, K. / 202, 203
Seman, G. / 88, 573, 574, 575, 576, 577
Sendo, F. / 28, 30, 112
Sessions, R.B. / 578
Seth, P. / 579
Sethi, V.S. / 456, 457, 458, 580, 938
Shah, K.V. / 581, 965
Shanmugam, G. / 242, 243, 582, 678
Shanmugaratnam, K. / 595, 781, 947, 991
Shantz, G.D. / 583
Shapiro, S.Z. / 584
Sharar, A. / 554
Sheffield, J.B. / 585, 977
Sherr, C.J. / 586, 587, 588, 658, 659, 758, 888, 907, 908, 909
Shibley, G.P. / 589
Shigematsu, T. / 159
Shih, M.S. / 667, 668
Shinedling, S.T. / 336, 337
Shlomai, Y. / 53, 751
Shope, T.C. / 324, 325
Shore, B. / 79
Showalter, S.D. / 258
Shows, T. / 231, 827
Shoyab, M. / 46, 47, 185, 186, 590, 591, 807
Shullenberger, C.C. / 992, 994, 1033
Sibal, L.R. / 32, 33, 34, 121, 735, 990
Siegler, R. / 592
Sigel, M.M. / 163, 377, 378
Silberstein, H. / 39, 593
Silva, D. / 789
Silverman, A.Y. / 864
Silverstone, A.E. / 892
Silvestre, D. / 182, 594
Simeone, T. / 200
Simonds, J.R. / 669

Simons, M.J. / 149, 595, 779, 780, 781, 787, 947, 991
Singh, M. / 596, 597
Singh, S. / 252, 886
Singh, V.N. / 596, 597
Sinkovics, J.G. / 331, 332, 598, 599, 600, 601, 654, 655, 992, 993, 994, 995, 1019, 1033, 1040
Sisillo, E. / 1014
Siwarski, D.F. / 405
Skeen, P.C. / 959
Skinner, G.R.B. / 494
Skurzak, H.M. / 356, 893
Smeraglia, R. / 1014
Smith, B.G. / 747
Smith, G.C. / 267, 320, 321, 875, 996
Smith, J.W. / 525
Smith, M.F. / 742, 819
Smith, M.H.D. / 1008
Smith, P.G. / 366, 490, 602, 954, 957
Smith, R.E. / 763, 905
Smith, R.G. / 211, 212, 213, 406, 527 528, 657
Smith, R.W. / 542
Smotkin, D. / 70
Smythers, G. / 944
Soares, N. / 284
Söderberg, G. / 871
Soeiro, R. / 603, 631
Sofranko, J. / 495
Sohier, R. / 796, 797
Somers, K. / 613
SooHoo, J. / 851, 925, 955
Sosin, H. / 207
Sottong, P. / 604
Souissi, T. / 291, 529, 605, 997
Soule, H.D. / 413, 414, 415, 416, 536, 547, 736, 737
Spahn, G. / 485, 496
Sparks, R.B. / 363
Spector, B. / 334, 883
Spergel, G. / 757
Spiegelman, S. / 123, 124, 125, 482, 567, 568, 606, 607, 608, 787, 926, 927, 984, 998
Spieler, L.E. / 900
Spiewak, J.E. / 869
Spira, G. / 999
Springer, E.L. / 254, 609, 610, 630
Stafford, K. / 604
Stavnezer, E. / 611
Stavnezer, J. / 611
Steele, R.W. / 175, 346, 876
Steeves, R. / 373, 612
Stehlin, J.S. / 886
Steiner, M. / 344, 1000, 1001
Steiner, M.R. / 613, 1026
Steiner, S. / 613, 614, 731, 1026
Stephens, R. / 615, 616
Stephenson, J.R. / 2, 3, 4, 35, 246, 247, 617, 618, 619, 620, 621, 660, 661, 722, 1022
Storb, R. / 96, 97, 98, 196, 235, 314, 347, 455, 622, 623, 624, 653, 662, 693, 831, 832, 986, 987, 988, 1002, 1003, 1004, 1005, 1017, 1018, 1023, 1024, 1025, 1034, 1035, 1036, 1037
Strand, M. / 72, 73, 310, 584, 612, 625, 626, 627, 628, 629, 718, 738
Strandberg, J.D. / 43, 44
Strobel, P.L. / 630
Sturm, M.M. / 31
Sugden, B. / 887, 1007
Suk, W.A. / 37, 496, 959
Sulzer, H.J. / 630
Sumaya, C.V. / 1008
Summers, M.R. / 469, 944, 945
Sundström, C. / 343
Suzuki, Y. / 311, 312
Sveda, M.M. / 603, 631
Svedmyr, E.A. / 632, 633, 634, 635, 1008
Swanson, M.H. / 229
Swen, S. / 529
Szakacs, J.E. / 636, 637
Szakacs, M.R. / 636, 637

Tagamets, M.A. / 258
Takasugi, M. / 638, 943
Tamerius, J.D. / 639
Tanaka, A. / 259, 452, 453, 640, 841, 1010
Tarro, G. / 300, 548, 641, 642, 643, 644, 898, 1011, 1012, 1013, 1014
Tavitian, A. / 359, 360, 462, 645, 894
Taylor, B.A. / 268
Taylor, G.A. / 923
Taylor, J.M. / 126, 646, 647, 1015
Tchen, P. / 462, 645
Teich, N.M. / 110, 111, 380
Tennant, R.W. / 260, 308, 470, 481, 504, 569, 648, 649, 650, 651, 713, 847, 1016
Teramoto, Y.A. / 105, 652

Terasaki, P.I. / 74, 75
Theuns, G. / 292, 858
Thomas, E.D. / 96, 97, 98, 235, 314, 347, 455, 622, 623, 624, 653, 692, 831, 832, 986, 1003, 1004, 1017, 1018, 1023, 1024, 1036, 1037
Thota, H. / 332, 654, 655, 993, 994, 1019
Thouless, M.E. / 494
Ti, M. / 656, 1020
Tilton, J. / 326, 327
Ting, R.C. / 213, 657, 967
Todaro, J.G. / 64, 65, 66, 67, 103, 278, 586, 587, 588, 658, 659, 758, 765, 776, 806, 874, 907, 908, 909, 989
Todd, D. / 646
Tomkins, G.M. / 766, 767, 784, 785
Toni, R. / 823
Torgerson, J. / 951
Traul, K. / 615, 616, 1021
Tronick, S.R. / 618, 620, 621, 660, 661, 1022
Tschudy, D.P. / 86
Tseng, J. / 554
Tsoi, M.S. / 662, 692, 1023, 1024, 1025, 1034, 1035
Tsuchida, N. / 663, 664, 665, 666, 667, 668
Tsukimoto, I. / 715
Tucker, T. / 1026
Tukei, P. / 797
Tulp, A. / 291
Twardzik, D. / 669, 723, 811, 1027

Uitendaal, / 291

Vaidya, A.B. / 150, 151, 152, 153, 362, 670, 671
Vaitkevicius, V.K. / 339
Valerio, M.G. / 114, 506
Vallee, B.L. / 739
Vanaman, I.C. / 763, 853
Van Blitterswijk, W.J. / 291, 292, 857, 858, 1028
Van der Maaten, M.J. / 823
Vance, J.C. / 672, 882, 1029
Van Griensven, L.J.L.D. / 529
Van Nie, R. / 292, 605, 673, 681, 682, 857, 997, 1030, 1031, 1032
VanVleck, R. / 484
Varmus, H.E. / 77, 78, 145, 646, 674, 675, 676, 677, 837, 838, 969
Veatch, B. / 915
Vecchio, G. / 242, 678
Verbeet, M. / 102, 291, 774, 1028
Veres-Molnar, Z. / 100
Verma, I.M. / 197, 225, 475, 679, 680, 1022
Vernon, M.L. / 533, 978
Verstraeten, A. / 605, 673, 681, 682, 997, 1030, 1031, 1032
Vidrine, J.G. / 15
Vigier, P. / 683, 740, 746, 970
Vogt, P.K. / 54, 164, 165, 166, 204, 205, 257, 306, 352, 400, 684, 685, 686, 808
Vonka, V. / 797

Wagner, S.H. / 397, 398, 399
Wahl, D.V. / 687
Wald, N. / 299
Wall, R. / 807
Wallen, W.C. / 114, 447, 506, 507, 688
Walter, L. / 58
Wang, C.-H. / 991, 994, 1032, 1033
Wang, L.H. / 689
Warren, R.P. / 988, 1034, 1035
Wars, I. / 172
Waters, L.C. / 690, 691, 712
Wee, G.B. / 595, 991
Wei, T. / 508
Weiden, P.L. / 347, 662, 692, 987, 1004, 1005, 1023, 1024, 1025, 1034, 1035, 1036, 1037
Weinbach, E.C. / 86
Weinberg, E. / 53, 69, 752, 753
Weinberg, R. / 70
Weiner, L.P. / 316
Weiss, F. / 336, 337
Weiss, R.A. / 686
Weisensee, M. / 915
Weissman, S.M. / 197
Wernet, O. / 1038, 1039
Wexler, I. / 388
White, J. / 382, 882
Whitmire, C.E. / 693
Whitten, H.D. / 356, 694, 893
Wigzell, H. / 632, 635
Wiener, F. / 344
Wildy, P. / 494
Wilkes, B. / 16
Williams, D.E. / 599, 600, 601, 994
Williams, D.R. / 304
Williams, E.H. / 135, 148, 695, 696
Williams, J.A. / 405
Wilsnack, R.E. / 629
Wilson, A.T. / 70
Wilson, G.L. / 64

Witkin, S. / 714
Wolf, H. / 795
Wolfe, L. / 141, 142, 187, 188, 448, 794, 815, 936
Wolter, J. / 369, 899
Woo, S.L.C. / 973
Wood, M.L. / 867
Woolf, D. / 615, 616
Wright, J. / 188, 697, 698, 1041, 1042
Wright, M. / 541
Wright, P.J. / 699, 1043
Wright, P.W. / 700, 701
Wright, S.E. / 1004
Wright, W.E. / 1044, 1045, 1046
Wu, A.M. / 212, 213, 427, 477, 500, 556, 657, 702, 703, 704, 705, 706, 707, 708, 709, 967, 1047
Wuu, K.D. / 533
Wybran, J. / 900
Wyke, J.A. / 686

Yajima, Y. / 534
Yamamoto, K. / 946
Yamashita, T. / 243, 710, 1048
Yamazaki, H. / 202, 203
Yang, C.S. / 711, 947
Yang, W.K. / 569, 651, 690, 691, 712, 713, 866, 867
Yaniv, A. / 714
Yap, J. / 779
Yata, J. / 715, 1049
Yates, J.W. / 59
Yee, C. / 932
Yeh, J. / 13, 14, 716, 717
Yohn, D.S. / 303, 404, 464, 465, 466, 561, 941
Yoshida, T. / 311, 312, 443
Yoshiki, T. / 310, 718
Young, H.A. / 949
Young, J. / 984
Young, L.J.T. / 1051
Young, M. / 277
Young, S. / 11
Yuhas, J.M. / 481
Yunis, E.J. / 208, 333, 733, 782, 880
Yurconic, M., Jr. / 288

Zachrau, M. / 79
Zajac, B.A. / 1052
Zamecnik, P.C. / 374, 510
Zavadova, H. / 797
Zeve, V.H. / 720
Zhdanov, V.M. / 446
Zimmerman, E.M. / 349
Zimmerman, J.E. / 418, 830
Zur Hausen, H. / 181, 795
Zusman, J. / 208

A. PAPERS PUBLISHED

1. Aaronson, S.A.: Cellular regulation of endogenous mouse type C RNA viruses. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

2. Aaronson, S.A. and Stephenson, J.R.: Cellular regulation of biologically distinguishable endogenous mouse type C RNA viruses. In: The Immune System: Genes, Receptors, Signals, Proc ICN-UCLA Symp Mol Biol, (E. Sercarz, A. Williamson and C.F. Fox, eds.) Academic Press, 1974, pp 441-445.

3. Aaronson, S.A. and Stephenson, J.R.: Differential cellular regulation of three distinct classes of type C RNA viruses endogenous to mouse cells. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

4. Aaronson, S.A., Stephenson, J.R. and Greenberger, J.S.: Cellular replication and the persistence of inducible RNA type C viruses. J Virol 13: 1404-1407, Sept. 1974.

5. Ablashi, D.V., Adamson, R.H., Rabin, H., et al: Chemotherapy of herpesvirus saimiri induced lymphoma and leukemia in owl monkeys. J Med Primatol 3: 68-72, 1974.

6. Ablashi, D.V., Armstrong, G.R., Easton, J.M., et al: Evaluation of effects of cytosine arabinaside on oncogenic herpesvirus saimiri in owl monkey kidney cells. Biomed 21: 57-60, 1974.

7. Ablashi, D.V., De Thé, G.B., Easton, J.M., Liabeuf, A., Levine, P.H., and Armstrong, G.R.: Antibodies to Epstein-Barr virus (EBV) antigens in sera of American Burkitt lymphoma patients. Biomedicine 20(4): 288-293, July 1974.

8. Ablashi, D.V., Goodheart, C.R., Armstrong, G.R.: Concentration of oncogenic herpesvirus by methyl alcohol precipitation. Appl Microbiol 27: 988-990, 1974.

9. Ablashi, D.V., Heine, U.: Morphological studies of herpesvirus in primate cell culture. J Med Primatol 3: 18-26, 1974.

10. Ablashi, D.V., Pearson, G.: Herpesvirus saimiri. A non-human primate model for herpesvirus-associated neoplasia of man. Int Workshop on Human Tumor Associated with Herpesvirus, Cancer Res 34: 1232-1236, 1974.

11. Ablashi, D.V., Young, S., Armstrong, G.R., et al: RNA-dependent DNA polymerase associated with a saimiri lymphoma cell line derived from a herpesvirus saimiri-induced lymphoma. Int J Cancer 13: 82-90, 1974.

12. Abramson, J.H.: Childhood experience and Hodgkin's disease in adults. Isr J Med Sci 10 (11): 1365-1370, 1974.

13. Ahmed, M., Herles, S., Yeh, J., Korol, W., Mayyasi, S. and Schidlovsky, G.: A new infectivity assay for the Mason-Pfizer monkey virus (M-PMV) and similar isolates. IX Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

14. Ahmed, M., Korol, W., Yeh, J., Schidlovsky, G. and Mayyasi, S.A.: Detection of Mason-Pfizer monkey virus infection using human KC cells carrying Rous sarcoma virus genome. J Natl Cancer Inst 53: 383-387, 1974.

15. Ahmed, M., Schidlovsky, G., Korol, W., Vidrine, J. and Cicmanec, J.L.: Occurrence of Mason-Pfizer monkey virus in healthy rhesus monkeys. Cancer Res 34: 3504-3508, Dec. 1974.

16. Aisenberg, A.C., Long, J.C., and Wilkes, B.: Chronic lymphocytic leukemia cells: Rosette formation and adherence to nylon fiber columns. J Natl Cancer Inst 52: 13-17, 1974.

17. Albrecht, T., St. Jeor, S.C., Funk, F.D. and Rapp, F.: Multiplicity reactivation of human cytomegalovirus inactivated by ultraviolet light. Int J Radiation Biol 26: 445-454, 1974.

18. Allen, P.T., Georgiades, J.A., Kurylo-Borowska, Z. and Dmochowski, L.: Enhancement of oncornavirus production by edeines. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

19. Allen, P.T., Miller, M.F., Bowen, J.M., East, J.L., Chan, J.C., Georgiades, J., Maruyama, K. and Dmochowski, L.: Reverse transcriptase inhibitor-normal in normal and neoplastic animal and human cell culture fluids. In: 1974 Annual Meeting of Am Soc for Microbiol, Vol. 282, Chicago, Ill., May 1974, p 247.

20. Allen, P.T., Newton, W.A., East, J.L., Maruyama, K., Bowen, J.M., Georgiades, J., Priori, E.S. and Dmochowski, L.: Molecular studies of cells derived from human solid tumors. In: Molecular Studies in Viral Neoplasia. Williams and Wilkins, Baltimore, 1974, pp 531-546.

21. Anderson, N.G., Holladay, D.W., Caton, J.E., Candler, E.L., Dierlam, P.J., Eveleigh, J.W., Ball, F.L., Holleman, J.W., Breillatt, J.P. and Coggin, J.H., Jr.: Searching for human tumor antigens. Cancer Res 34: 2066-2076, Aug. 1974.

22. Anderson, P.N., Klein, D.L., Bias, W.B., Mullins, G.M., Burke, P.J. and Santos, G.W.: Cell-mediated immunological reactivity of patients and siblings to blast cells from adult acute leukemia. Isr J Med Sci 10(8): 1033-1051, 1974.

23. Anderson, P.N., Klein, D.L. and Santos, G.W.: Macrophage migration inhibition factor (MIF) production in response to leukemia associated antigens. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

24. Aoki, T.: A review on antigen of RNA oncogenic viruses. II. Medical Chugai 27: 311-318, 1974. (In Japanese).

25. Aoki, T.: Host immune response to tumors. III. Medical Chugai 27: 351-356, 1974. (In Japanese).

26. Aoki, T.: Reclassification and reconsideration of antigens on surfaces of tumors. Medical Chugai 27(8): 11-18, 1974. (In Japanese).

27. Aoki, T. and Chang, S.S.: Type C RNA viruses: Diverse immunological and etiological roles. In: Advances in the Biosciences 12 (G. Raspe and S. Bernhard, eds.) Pergamon Press-Vieweg, 1974, pp 597-603.

28. Aoki, T., Chieco-Bianchi, L., Plata, E.J., Sendo, F., Hollis, V.W., Jr. and Kudo, T.: Host immune response to virus-induced tumors: Some recent concepts. Immunol of Cancer Prog Exp Tumor Res 19: 23-36 1974.

29. Aoki, T. and Hanafusa, H.: Cell surface antigens of type C RNA viruses as markers for detection of viruses. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 441-442.

30. Aoki, T., Kvedar, J.P., Kudo, T., Plata., E.J., Sendo, F. and Hollis, V.W., Jr.: Enhancement of host immune response to cell surface antigens by the extract of Streptococcus hemolyticus. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 237-240.

31. Aoki, T., McKenzie, I.F.C., Sturm, M.M. and Liu, M.: Distribution of the alloantigen Ly-4.2 on murine B-cells. Immunogenetics 1(3): 291-296, Oct. 1974.

32. Aoki, T. and Sibal, L.R.: Immunology of virus induced leukemias. In: Handbook of Hematology (Japan Hematological Soc, ed.) Maruzen Publishers, Tokyo, Japan, 1974.

33. Aoki, T. and Sibal, L.R.: RNA oncogenic virus-associated antigens. In: Cancer: A Comprehensive Treatise (F.Becker, ed.) Plenum Press, 1974.

34. Aoki, T. and Sibal, L.R.: Surface antigens associated with RNA type C viruses. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

35. Aoki, T., Stephenson, J.R., Aaronson, S.A. and Hsu, K.C.: Surface antigens of mammalian sarcoma virus-transformed nonproducer cells. Proc Natl Acad Sci USA 71: 3445-3449, Sept. 1974.

36. Arnold, J. and Bradley, R.: Recovery of minute samples after ultracentrifugation. J Cell Biol 63(2): 10a, Nov. 1974, (abstract).

37. Arnstein, P., Levy, J.A., Oshiro, L.S., Price, P.J., Suk, W.A. and Lennette, E.: Recovery of murine xenotropic type C virus from C57/Leaden mice. J Natl Cancer Inst 53: 1787-1792, 1974.

38. Arthur, L.O., et al: Characterization of DNA polymerase from mouse mammary tumor virus produced in tissue culture. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

39. August, J.T. and Silberstein, H.: A virion protein kinase: Identification as a virus specified protein. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

40. Aupoix, M.C., Biquard, J.M. and Cachard, A.: Cell surface antigen induced by avian tumor viruses in hamster cells transformed by a temperature-sensitive mutant of Rous sarcoma virus. Int J Cancer 14(5): 611-616, Nov. 1974.

41. Aurelian, L.: Persistence and expression of the herpes simplex virus type 2 genome in cervical tumor cells. In: 1975 Year Book of Pathology and Clinical Pathology, April, 1975.

42. Aurelian, L.: The "viruses of love" and cancer. Am J Med Tech 40: 496-512, 1974.

43. Aurelian, L. and Strandberg, J.D.: Biologic and immunologic comparison of two HSV-2 variants: One an isolate from cervical tumor cells. Arch Gesamte Virusforsch 45: 27-38, 1974.

44. Aurelian, L., Strandberg, J.D. and Marcus, R.L.: Neutralization, immunofluorescence and complement fixation tests in the identification of antibody to a herpesvirus type 2 induced tumor specific antigen in sera from squamous cervical carcinoma. Prog Exp Tumor Res 19: 165-181, 1974.

45. Baltimore, D.: Tumor viruses: 1974. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

46. Baluda, M.A., Shoyab, M., Markham, P.D., Evans, R.M. and Drohan, W.N.: Base sequence complexity of 35S avian myeloblastosis virus RNA determined by molecular hybridization kinetics. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

47. Baluda, M.A., Shoyab, M., Evans, R., Markham, P.D. and Ali, M.: Quantitative and qualitative differences in DNA complementary to avian myeloblastosis virus between normal and leukemic chicken cells. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R. M. Dutcher, eds.) Univ of Tokyo Press, 1975.

48. Bandyopadhyay, A.K.: Partial purification and properties of DNA polymerases from JLS-V10 cells. Biochem Biosphy 166: 83-93, 1975.

49. Bandyopadhyay, A.K.: Properties of RNA-dependent DNA polymerase of Rauscher leukemia virus. Fed Proc 34(3) April 1975 (abstract).

50. Bandyopadhyay, A.K.: Purification and properties of nuclear and cytoplasmic DNA polymerases from JLS-V9 cells. Arch Biochem Biophys 166: 72-82, 1975.

51. Barahona, H., Garcia, F.G., Melendez, L.V. and Hunt, R.D.: A procedure for the isolation of herpesvirus saimiri, other lymphocytic associated viruses and blood parasites from monkeys living in the wild. J Med Primatol 3: 370-376, Dec. 1974.

52. Becker, Y. and Asher, Y.: In vitro synthesis of DNA in nuclei isolated from herpes simplex virus infected cells, untreated and treated with metabolic inhibitors. Virology 63: 209-220, 1975.

53. Becker, Y., Shlomai, Y., Weinberg, E., Ben Zeev, A. and Olshevsky, U.: Integration of EBV DNA into cellular DNA of Burkitt lymphoblasts (Raji cell-line). Isr J Med Sci 10: 1454-1457, 1974.

54. Beemon, K., Duesberg, P.H. and Vogt, P.K.: Evidence for crossing-over between avian tumor viruses based on analysis of viral RNAs. Proc Natl Acad Sci USA 71(10): 4254-4258, Oct. 1974.

55. Bekesi, J.G. and Holland, J.F.: Combined chemotherapy and immunotherapy of transplantable and spontaneous murine leukemia in DBA/2 and AKR mice. In: Recent Results in Cancer Research (G. Mathe and R. Weiner, eds.) Springer-Verlag, Berlin, New York, 1974, 47: 357.

56. Bekesi, J.G., Roboz, J.P. and Holland, J.F.: Humoral and cellular immunity in mice immunized with syngeneic tumor cells. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, p 229 (abstract).

57. Bekesi, J.G., Roboz, J.P. and Holland, J.F.: Immunotherapy of tumors (spontaneous or transplantable) in mice. 2nd Congress of Immunology, Brighton, England, 1974 (abstract).

58. Bekesi, J.G., Roboz, J.P., Walter, L. and Holland, J.F.: Stimulation of specific immunity against cancer by neuraminidase-treated tumor cells. Symp on Neuraminidase, Vol 54 (K. Heide, R.H. Seiler and H.G. Schwick, eds.) Behring Inst Mitteilungen, Marburg, 1974, p 309.

59. Bekesi, J.G., Roboz, J.P., Yates, J. and Holland, J.F.: Neuraminidase-treated leukemic cells in combination with cytoreductive therapy in man and experimental animals. Symp on Specific Immunostimulation with Modified Leukemic Cells, XV Cong Int Soc Hematol, Jerusalem, Sept. 1974 (abstract).

60. Ben-Bassat, H., Goldblum, N., Manny, N. and Sachs, L.: Mobility of concanavalin A receptors on the surface membrane of lymphocytes from normal persons and patients with chronic lymphocytic leukemia. Int J Cancer 14: 367-371, 1974.

61. Benirschke, K., Bogart, M.H., McClure, H.M. and Nelson-Rees, W.A.: Fluorescence of the trisomic chimpanzee chromosomes. J Med Primatol 3: 311-314, 1974.

62. Benjamin, T. and Goldman, E.: Indirect complementation of a non-transforming mutant of polyoma virus. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

63. Bentvelzen, P.: Host-virus interactions in murine mammary carcinogenesis. Biochim et Biophys Acta (Reviews on Cancer) 355: 236-259, 1974.

64. Benveniste, R.E., Heinemann, R., Wilson, G.L., Callahan, R. and Todaro, G.J.: Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. J Virol 14: 56-67, July 1974.

65. Benveniste, R. E. and Todaro, G.J.: Evolution of C-type viral genes. Inheritance of exogenously acquired viral genes. Nature 252: 456-459, Dec. 1974.

66. Benveniste, R. E. and Todaro, G.J.: Evolution of type C viral genes. I. Nucleic acid from baboon type C virus as a measure of divergence among primate species. Proc Natl Acad Sci USA 71: 4503-451B, Nov. 1974.

67. Benveniste, R.E. and Todaro, G.J.: Multiple divergent copies of endogenous type C virogenes in mammalian cells. Nature 252: 170-173, Nov. 1974.

68. Benyesh-Melnick, M., Schaffer, P.A., Courtney, R.J., Esparza, J. and Kimura, S.: Viral gene functions expressed and detected by temperature-sensitive mutants of herpes simplex virus. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

69. Ben Zeev, A., Weinberg, E. and Becker, Y.: Isolation of herpes simplex virus DNA from virions and infected cells by electrophoresis in polyacrylamide gels. J Gen Virol 25: 63-73, 1974.

70. Besmer, P., Smotkin, D., Haseltine, W., Fan, H., Wilson, A.T., Paskind, M., Weinberg, R. and Baltimore, D.: Mechanism of induction of RNA tumor viruses by halogenated pyrimidines. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

71. Bhattacharyya, J., Gallagher, R.E. and Gallo, R.C.: DNA polymerases in normal human blood platelets. 17th Annual Meeting of Am Soc Hematol, Atlanta, Ga., Dec. 1974, p 55 (abstract).

72. Bilello, J.A., Rogart, W. and Strand, M.: Characterization of murine type C virus interference as a property of the major virus envelope glycopeptide. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

73. Bilello, J.A., Strand, M. and August, J.T.: Murine sarcoma virus gene expression: Transformants which express viral envelope glycoprotein in the absence of the major internal protein and infectious particles. Proc Natl Acad Sci USA 71: 3234-3438, 1974.

74. Billing, R., Terasaki, P.I.: Human leukemia antigen I. Production and characterization of antisera. J Natl Cancer Inst 53: 1635-1638, 1974.

75. Billing, R., Terasaki, P.I.: Human leukemia antigen II. Purification. J Natl Cancer Inst 53: 1639-1643, 1974.

76. Biquard, J.M.: Agglutination by concanavalin A of normal chick embryo fibroblasts treated by 5-Bromodeoxyuridine (Brdu). J Cell Physiol 84(3): 459-462, Dec. 1974.

77. Bishop, J.M., Quintrell, N., Medeiros, E., and Varmus, H.E.: Of birds and mice and men: Comments on the use of animal models and molecular hybridization in the search for human tumor viruses. Cancer 34(4): 1421-1426, Oct. 1974.

78. Bishop, J. M., and Varmus, H. E.: The molecular biology of RNA tumor viruses. In: Cancer: A Comprehensive Treatise, Vol 2 (I.F. Becker, ed.) Plenum Press, 1975, p 47.

79. Black, M., Zachrau, R.E., Shore, B., Moore, D.H., and Leis, H.P., Jr.: Prognostically favorable immunogens of human breast cancer tissue: Antigenic similarity to murine mammary tumor virus. Cancer 35: 121-128, Jan. 1975.

80. Bogden, A.E., Cobb, W.R., Ahmed, M., Alex, S. and Mason, M.M.: Oncogenicity of the R-35 rat mammary tumor virus. J Natl Cancer Inst 53: 1073-1077, Oct. 1974.

81. Bogden, A.E., Cobb, W.R., Cate, C.C., Esber, H.J., Kuo, E.Y.H. and Alex, S.A.: Inhibition of X-irradiation-induced mammary tumorigenesis by 17β-Estradiol. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

82. Bolognesi, D.P.: Structural components of RNA tumor viruses. Adv Virus Res 20: 313-359, 1974.

83. Bolognesi, D.P., Hüper, G., Green, R.W., and Graf, T.: Biochemical properties of oncornavirus polypeptides. Biochim Biophys Acta Revs Cancer 355, 220-235, 1974.

84. Bolognesi, D.P., Leis, J.P., Grant, J.P. and Schäfer, W.: Properties of oncornavirus structural components and their expression in the host cell. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

85. Bone, D. R., and Courtney, R. J.: A temperature-sensitive mutant of herpes simplex virus type 1 defective in the synthesis of the major capsid polypeptide. J Gen Virol 24: p 17-27, July 1974.

86. Bonkowsky, H.L., Tschudy, D.P., Weinbach, E.C., Ebert, P.S. and Doherty, J.M.: Porphyrin synthesis and mitochondrial respiration in acute intermittent porphyria: Studies using cultured human fibroblasts. J Lab & Clin Med 85: 93-102, Jan. 1975.

87. Bowen, J.M.: Molecular studies of the role of viruses in human neoplasia. Cancer Bulletin 26(4): 65-68, July-Aug. 1974.

88. Bowen, J.M., East, J.L., Allen, P.T., Maruyama, K., Priori, E.S., Georgiades, J., Chan, J.C., Miller, M.F., Seman, G., and Dmochowski, L.: Comparative morphology, immunology, and biochemistry of viruses associated with neoplasia in animals and man. In: Viruses, Evolution and Cancer - Basic Considerations, Proc 2nd Int Conf on Comp Virol, Mont Gabriel, Quebec, Aug. 22-24, 1974 (E. Kurstak and K. Maramorosch, eds.) Academic Press, New York, 1974, pp 403-426.

89. Bowen, J.M., McBride, C.M., Miller, M.F., Hersh, E., and Dmochowski, L.: Tumor-cell associated nucleolar antigens and disease prognosis in patients with melanoma. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 104-105, (abstract).

90. Bowling, M., Deepak, J., Bandyopadhyay, A.K.: Separation of RNase H activity from RNA-dependent DNA polymerase of Rauscher leukemia virus. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

91. Brack, M., Kalter, S.S., Heberling, R.L., Eichberg, J.: Herpesvirus pathology in Cercopithecus aethiops. J Med Primatol 3: 325-333, 1974.

92. Brandchaft, P.B. and Boone, C.W.: Extraction of Gross (G) surface antigens from AKR mouse lymphoma cells with 3M potassium chloride. J Natl Cancer Inst 53: 1079-1083, Oct. 1974.

93. Brandchaft, P.B. and Boone, C.W.: Increase in Gross (G) antigen sites on the surface of AKR virus-induced rat lymphoma cells after treatment with trypsin. J Immunol 113 (1): 94-102, July 1974.

94. Brown, B.L. and Nagle, S.C.: A simplified method for titering Epstein-Barr virus by super-infection of Raji cells. In Vitro 9: 389, 1974.

95. Buckley, P.M., Kawakami, T.G., and DePaoli, A.: Type C virus in a gibbon colony with a high incidence of hematopoietic neoplasia. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N. Y.

96. Buckner, C.D., Briggs, R., Clift, R.A., Fefer, A., Funk, D.D., Glucksberg, H., Neiman, P.E., Storb, R. and Thomas, E.D.: Intermittent high dose cyclophosphamide treatment of stage III ovarian carcinoma. Cancer Chemother Rep 58: 697-703, 1974.

97. Buckner, C.D., Clift, R.A., Fefer, A., Funk, D.D., Glucksberg, H., Neiman, P.E., Paulsen, A., Storb, R. and Thomas, E.D.: High dose cyclophosphamide for the treatment of metastatic testicular neoplasms. Cancer Chemother Rep 58: 709-714, 1974.

98. Buckner, C.D., Clift, R.A., Fefer, A., Neiman, P.E., Storb, R. and Thomas, E.D.: Treatment of blastic transformation of chronic granulocytic leukemia by high dose cyclophosphamide, total body irradiation and infusion of cryopreserved autologous marrow. Exp Hematol 2: 138-146, 1974.

99. Buehring, G.C. and Hackett, A.J.: Human mammary tumor cell lines: Identity evaluation by ultrastructure. J Natl Cancer Inst 53(3): 621-629, Sept. 1974.

100. Buettner, W., Veres-Molnar, Z. and Green, M.: Isolation of DNA strand-specific early messenger RNA species in human adenovirus 2 infected cell. Proc Natl Acad Sci USA, 71: 2951-2955, 1974.

101. Calafat, J., Buys, F., Hageman, Ph.C., Links, J., Hilgers, J. and Hekman, A.: Distribution of virus particles and mammary tumor virus antigens in mouse mammary tumors, transformed BALB/c mouse kidney cells, and GR ascites leukemia cells. J Natl Cancer Inst 53(4): 977-992, Oct. 1974.

102. Calafat, J., Hageman, Ph., Hilgers, J., Verbeet, M. and Nusse, R.: Ultrastructural distribution and redistribution of mammary tumor virus antigens and Thy. 1.2 antigens on GR ascites leukemia cells. In: Proc 9th Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

103. Callahan, R., Benveniste, R.E., Lieber, M.M. and Todaro, G.J.: Nucleic acid homology of murine type C viral genes. J Virol 14: 1394-1403, Dec. 1974.

104. Canaani, E. and Duesberg, P.H.: The preferred role of 60-70S avian tumor virus RNA among natural RNAs as template for DNA polymerase of Rous sarcoma virus (RSV). In: Molecular Studies in Viral Neoplasia, 25th Annual Symp on Fundamental Cancer Res, 1972, Univ of Texas, M.D. Anderson Hosp and Tumor Inst, Houston, Texas, Williams and Wilkins Co., Baltimore, Md., 1974, pp 97-114.

105. Cardiff, R.D., Puentes, M.J., Teramoto, Y.A. and Lund, J.K.: Structure of the mouse mammary tumor virus: characterization of bald particles. J Virol 14(5): 1293-1303, Nov. 1974.

106. Cejka, J., Peterson, W.D. Jr., Belamaric, J., and Kithier, K.: In vitro synthesis of B_2 microglobulin by human fetal tissues. Dev Biol 43: March 1975.

107. Chan, J.C., East, J.L., Bowen, J.M., and Dmochowski, L.: Characterization of a type C virus produced by a BALB/c mouse mammary gland cell culture. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

108. Charman, H.P., Kim, N., and Gilden, R.V.: Radioimmunoassay for the major structural protein of hamster type C viruses. J Virol 14: 910-917, Oct. 1974.

109. Charney, J., Moore, D.H., Holben, J.A., and Cody, C.M.: Rejection of syngeneic mammary tumor cells following immunization with killed mouse mammary tumor virus (MuMTV). Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

110. Chattopadhyay, S.K., Rowe, W.P., Teich, N.M. and Lowy, D.R.: Definitive evidence that the murine C-type virus inducing locus Akv-1 is viral genetic material. Proc Nat Acad Sci USA 72, March 1975.

111. Chattopadhyay, S.K., Lowy, D.R., Teich, N.M. Levine, A.S. and Rowe, W.P.: Qualitative and quantitative studies of AKR-type murine-leukemia-virus (MLV) sequences in DNA of high-, low-, and non-virus-yielding mouse strains. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

112. Chieco-Bianchi, L., Colombatti, A., Collavo, D., Sendo, F., Aoki, T., and Fischinger, P.J.: Tumor induction by murine sarcoma virus in AKR and C58 mice: Reduction of tumor regression associated with appearance of Gross leukemia virus pseudotypes. J Exp Med 140: 1162-1179, Nov. 1974.

113. Cicmanec, J.L., and Campbell, A.K.: Breeding owl monkeys (Aotus trivirgatus) in a laboratory environment. Preliminary observations. Proc 25th Annual Mtg of Am Assoc Lab Anim Sci, Cincinnati, Ohio, Oct. 1974, (abstract).

114. Cicmanec, J.L., Loeb, W.F., Neubauer, R.H., Wallen, W.C., Rabin, H. and Valerio, M.G.: Clinical and hematologic changes observed in marmosets (Saguinus spp.) inoculated with an oncogenic type C virus. Proc 25th Annual Meeting Am Assoc Lab Anim Sci, Cincinnati, Ohio, Oct. 1974, (abstract).

115. Cicmanec, J.L., Neva, F.A., McClure, H.M., and Loeb, W.F.: Accidental infection of laboratory-reared Macaca mulatta with Trypanosoma cruzi. Lab Anim Sci 24(5): 783-787, Oct. 1974.

116. Coggin, J.H. Jr., Ambrose, K.R., Candler, E.L., and Hannon, W.H.: An evaluation of the isotopic antiglobulin assay and the cytostatic assay for detecting SV40 tumor immunity. Isr J Med Sci 10:904-912, Aug. 1974.

117. Coggin, J.H., Jr., Ambrose, K.R., Dierlam, P.J., and Anderson, N.G.: Proposed mechanisms by which autochthonous neoplasms escape immune rejection. Cancer Res 34: 2092-2101, Aug. 1974.

118. Coggin, J.H., Jr., and Anderson, N.G.: Cancer, differentiation, and embryonic antigens: Some central problems. Adv Cancer Res 19: 105-165, 1974.

119. Coggin, J.H., Jr. and Anderson, N.G.: Embryonic and fetal antigens in cancer cells. In: Developmental Aspects of Carcinogenesis and Immunity: 32nd Symp Soc Dev Biol (T. King, ed.) Academic Press, New York, 1974, pp 173-185.

120. Cohen, M.H., Bernstein, A.D., and Levine, P.H.: Hematological and serological effects of Rauscher leukemia virus and Epstein-Barr virus of immunosuppressed newborn sub-human primates. Oncology 29(5): 353-364, 1974.

121. Cohen, M.H., Sibal, L.R. and Fink, M.A.: Relative importance of viral and neoantigens in cytotoxic reaction against murine leukaemia cells. Immunology 26: 37-48, 1974.

122. Colcher, D., Schochetman, G., and Schlom, J.: Mason-Pfizer monkey virus (MPMV): A horizontally transmitted oncornavirus of Rhesus monkeys. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

123. Colcher, D., Spiegelman, S., and Schlom, J.: Relationship of Mason-Pfizer virus to human breast cancer. Annual Mtg Breast Cancer Task Force, 1975, p 49, (abstract).

124. Colcher, D., Spiegelman, S. and Schlom, J.: Sequence homology between MPMV cDNA and the RNA of human malignant breast tumors: IX Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, p 79, (abstract).

125. Colcher, D., Spiegelman, S., and Schlom, J.: Sequence homology between the RNA of Mason-Pfizer monkey virus and the RNA of human malignant breast tumors. Proc Natl Acad Sci USA 71: 4975-4979, 1974.

126. Cordell-Stewart B., Taylor, J.M., Rohde, W., Goodman, H.M., and Bishop, J.M.: Transfer RNAs of Rous sarcoma virus and the initiation of DNA synthesis by viral reverse transcriptase. In: Proc 1974 Abraham Flexner Symp on Viral Transformation and Endogenous Viruses (A. Kaplan, ed.) Academic Press, New York, 1974, pp 117-135.

127. Courtney, R.J. and Benyesh-Melnick, M.: Differential effect of 2-deoxy-D-glucose on glycoproteins synthesized by herpes simplex virus types 1 and 2. Intervirology 2: 120-127, 1974.

128. Courtney, R.J. and Benyesh-Melnick, M.: Isolation and characterization of a large molecular-weight polypeptide of herpes simplex virus type I. Virology 62: 539-551, 1974.

129. Croce, C.M., Huebner, F.K., Girardi, A.J. and Koprowski, H.: Genetics of cell transformation by Simian Virus 40. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

130. Croce, C.M., Huebner, K., Girardi, A.J., Koprowski, H.: Rescue of defective SV40 from mouse-human hybrid cells containing human chromosome C-7. Virology 60: 276-281, 1974.

131. Croker, B.P., Jr., Del Villano, B.C., Jensen, F.C., Lerner, R.A., and Dixon, F.J.: Immunopathogenicity and oncogenicity of murine leukemia viruses. I. Induction of immunologic disease and lymphoma in (BALB/c x NZB)F1 mice by Scripps leukemia virus. J Exp Med 140: 1028-1048, 1974.

132. Daniel, M.D., Melendez, L.V. and Hunt, R.D.: New Zealand white rabbits as a model for malignant lymphoma induced by herpesvirus saimiri. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, p 84, (abstract).

133. Daniel, M.D., Melendez, L.V. and Hunt, R.D., King, N.W., Anver, M., Fraser, C.E.O., Barahona, H.H. and Baggs, R.B.: Herpesvirus saimiri VII. Induction of malignant lymphoma in New Zealand white rabbits. J Natl Cancer Inst 53: 1803-1807, Dec. 1974.

134. Davis, J., Gilden, R.V. and Oroszlan, S.: Multiple species-specific and interspecific antigenic determinants of a mammalian type C RNA virus internal protein. Immunochemistry 12: 67-72, Jan. 1975.

135. Dean, A.G., Williams, E.H. and Hilgers, F.: Epidemiology of Burkitt's lymphoma in West Nile, Uganda. Am Soc Trop Med & Hygiene Mtgs, Nov 1974, (abstract).

136. Deepak, J., Comer, J., Bowling, J., Dobbs, J., Aldenderfer, D.H., Fish, D.C. and Bandyopadhyay, A.K.: Simultaneous purification of RNA-dependent DNA polymerase and gs-antigen from Rauscher leukemia virus. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

137. Deinhardt, F.: Antigens associated with DNA virus induced tumors. In: Progress in Immunology II. (L. Brent and J. Holborow, eds.), North Holland Publishing Co., 1974, pp 271-284.

138. Deinhardt, F.: Introduction to virus-caused cancer: Type C virus. Cancer 34(4): 1363-1367, Oct. 1974.

139. Deinhardt, F.: Nonhuman primates in cancer research. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

140. Deinhardt, F.: Oncogenic herpesviruses in species other than owl monkeys. A review. J Med Primatol 3(1): 79-88, 1974.

141. Deinhardt, F., Falk, L. and Wolfe, L.: Simian herpesviruses and neoplasia. In: Advances in Cancer Research 19 (G. Klein and S. Weinhouse, eds.) Academic Press, 1974, pp 167-205.

142. Deinhardt, F., Wolfe, L., Falk, L., Johnson, T., Johnson, D. and Massey, R.: Immunological control of virus-induced tumors in primates. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 574-584.

143. Del Villano, B.C., Croker, B.P., and Dixon, F.J.: Characterization of oncornavirus isolated from NZB lymphoblastoid cells. I. Host range and leukemogenic potential. Fed Proc 34(3) April 1975 (abstract).

144. Del Villano, B.C., Nave, B., Croker, B.P., Lerner, R.A. and Dixon, F.J.: The oncornavirus glycoprotein gp69/71 : A constituent of the surface of normal and malignant thymocytes. J Exp Med, Jan 1975.

145. Deng, C.T., Boettiger, D., MacPherson, I., and Varmus, H.E.: Expression of virus-specific DNA in revertants of Rous sarcoma virus-transformed BHK-21 cells. Virology 62(2): 512-521, Dec 1974.

146. Desai, L.S. and Livingston, D.M.: Studies on the type C viral replication defect in MSV-transformed rat nonproducer variant clones. Virology 62: 337-343, 1974.

147. De Thé, G., Ablashi, D.V., Favre, M.Cl., Mourali, N. and Ellouz, R.: Presence of EBV nuclear antigen in nasopharyngeal carcinoma (NPC) biopsies and their derived cultures. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 99-103.

148. De Thé, G., Geser, A. and Williams, E.H.: EB virus and treatment of lymphomas. Br Med J 4: 466, 1974.

149. De Thé, G. and Simons, M.J.: Nasopharyngeal carcinoma: Evidence for viral and immunogenetic factors. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

150. Dion, A.S. Vaidya, B.A. and Fout, G.S.: Cation preferences for poly(rC).oligo(dG) - directed DNA synthesis by RNA tumor viruses and human milk particulates. Cancer Res 34: 3509-3515, Dec 1974.

151. Dion, A.S., Vaidya, A.B., Fout, G.S. and Moore, D.H.: Comparative studies of isolated RNA-directed DNA polymerase(s) from milk-borne B type RNA tumor viruses and human milk particulates. Proc XIth Int Cancer Congress, Florence, Italy, Oct 1974 (abstract).

152. Dion, A.S., Vaidya, A.B., Fout, G.S. and Moore, D.H.: Isolation and characterization of RNA-directed DNA polymerase from a B-type RNA tumor virus. J Virol 14: 40-46, July 1974.

153. Dion, A.S., Vaidya, A.B., Ling-Tsang, A., Heubel, G.A., Fout, G.S. and Moore, D.H.: Intracellular processing of MuMTV-related RNA in murine mammary glands and tumors and human breast cancer. IX Meeting on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974 (abstract).

154. Dixon, F.J., Croker, B.P., Del Villano, B.C., Jensen, F.C. and Lerner, R.A.: Oncornavirus infection and "auto" immune complex disease of mice. In: Progress in Immunology II, Vol 5 (L. Brent and J. Holborow, eds.) North-Holland/American Elsevier, Amsterdam, New York, 1974, pp 49-55.

155. Dmochowski, L. and Bowen, J.M.: Current trends in basic immunology as applied to the problem of human neoplasia. Am J Clin Pathol 62(2): 167-172, Aug 1974.

156. Dmochowski, L., Bowen, J.M., Myers, B., Priori, E.S., Dodson, M.L. and Miller, M.G.: Comparative studies of type B and type C oncornaviruses. Proc Southwest Section Am Assoc for Cancer Res, Nov 8-9, 1974, New Orleans, La, pp 49-50 (abstract).

157. Dmochowski, L., Georgiades, J., East, J.L., Allen, P.T. and Bowen, J.M.: Virus retrieval studies in human neoplasia. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

158. Dmochowski, L., Hoshino, M., Miller, M.F., Priori, E.S., Hales, R.L. and Bowen, J.M.: Further studies on immunologic cross-reactivity between mouse and human breast cancer. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 301-302 (abstract).

159. Dmochowski, L., Hoshino, M., Shigematsu, T., Hiraki, S. and Priori, E.S.: Studies of oncornavirus by immunoferritin and immunoperoxidase electron microscopy. In: Viral Immunodiagnosis (E. Kurstak and R. Morisset, eds.) Academic Press, New York, N.Y., Nov 1974, pp 183-213.

160. Dmochowski, L., Miller, M.F., Priori, E.S., Bowen, J.M., Dodson, M.L. and Scanlon, M.: Immunological studies on mammary cancer in animals and man. In: Proc IX Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, p 50 (abstract).

161. Dmochowski, L., Miller, M.F., Priori, E.S., Dodson, M.L., Scanlon, M. and Bowen, J.M.: Immunological studies on mammary cancer in animals and man. Proc Southwest Section Am Assoc for Cancer Res, Nov 8-9, 1974, New Orleans, La., p 5-6 (abstract).

162. Dorf, M., Lilly, F., Benacerraf, B.: Characterization of a new intra-H-2 recombinant. Separation of the Ir-RF and Ir-GLT genes. J Exp Med 140: 859-864, Sept. 1974.

163. Dorizzi, M., Lopez, D.M., Ortiz-Muniz, G. and Sigel, M.M.: Differences in mitogenic responses of lymphocytes from normal and tumor-bearing mice in the presence of fetal calf (FCS) and rabbit sera (RS). Fed Proc 34(3) April 1975, (abstract).

164. Duesberg, P., Beemon, K., Lai, M. and Vogt, P.K.: Recombinants of avian tumor viruses: An analysis of their RNA. In: Mechanisms of Virus Disease, Vol I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 287-302.

165. Duesberg, P., Beemon, K., Lai, M. and Vogt, P.K.: Recombinants of avian RNA tumor viruses: Characteristics of the virion RNA. In: Viral Transformation and Endogenous Viruses. (A.S. Kaplan, ed.) Academic Press, New York-San Francisco, 1974, pp 137-153.

166. Duesberg, P., Vogt, P.K., Beemon, K. and Lai, M.: Avian RNA tumor viruses: Mechanism of recombination and complexity of the genome. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

167. Duff, R., Kreider, J.W., Levy, B.M., Katz, M. and Rapp, F.: Comparative pathology of cells transformed by herpes simplex virus type 1 or type 2. J Natl Cancer Inst 53: 1159-1164, 1974.

168. Duff, R. and Rapp, F.: Herpes simplex viruses. Proc Am Cancer Soc and Natl Cancer Inst Natl Conf on Virology and Immunology in Human Cancer, N.Y., N.Y., Nov. 29-Dec. 1, 1973, p 1353, (abstract).

169. Dunkel, V.C., Bast, R.C., Gerwin, B.I., Heine, U., Cottler-Fox, M. and Borsos, T.: Presence of A-type and absence of C-type particles in a chemically induced guinea pig hepatoma. J Natl Cancer Inst 53(2): 591-593, Aug. 1974.

170. Dux, A. and Muhlbock, O.: Mammary tumour incidence under different hormonal conditions in the BIMA strain, a new inbred strain derived from a cross between the C3H and the C57BL strains. In: Proc IX Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

171. East, J.L., Knesek, J.E., Chan, J.C. and Dmochowski, L.: Sequence homology of mammalian RNA tumor viruses. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

172. Ebert, P.S., Wars, I., and Buell, D.N.: Effect of chemotherapeutic agents on viral DNA polymerase and erythroid differentiation of Friend leukemia cells. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

173. Eckner, R.J., Priori, E.S., Mirand, E.A., and Dmochowski, L.: ESP-1 helper virus: Characterization of type-specific envelope specificities distinct from those of the murine leukemia viruses. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

174. Eckner, R.J., Priori, E.S., Mirand, E.A., and Dmochowski, L.: Studies on the biological and antigenic properties of ESP-1 type C virus particles. Cancer Res 34(10): 2521-2529, Oct. 1974.

175. Eichberg, J.W., Steele, R.W., Kalter, S.S., McCullough, B., and Kniker, W.T.: T and B lymphocytes in nonhuman primates and effects of thymus in vitro. Fed Proc 34(3) April 1975, (abstract).

176. Elkins, W.L., Hellström, I. and Hellström, K.E.: Transplantation tolerance and enhancement: Concepts and questions. Transplantation 18, 38-45, 1974.

177. Emanoil-Ravicovitch, R., Larsen, C.J., Robert-Robin, J., Bazilier, M. and Mauchauffe, M.: Study of low molecular weight RNA in a murine oncornavirus. Arch Int Physiol Biochim 82: 771, 1974, (abstract).

178. Emanoil-Ravicovitch, R., Robert-Robin, J., Bazilier, M. and Boiron, M.: Murine oncornavirus high-molecular-weight RNA structure thermal stepwise dissociation of 70S M-MSV (MLV) to subunits and low-molecular-weight associated RNAs. J Virol, Oct. 1974.

179. Emanoil-Ravicovitch, R., Sallé, M., Robert-Robin, J., Auriol, L. and Périés, J.: Characterization of the ribonucleic acids of a simian oncornavirus SSV_1. Comptes Rendus Acad Sci (série D), 280: 681, 1975.

180. Epstein, A. L., and Kaplan, H. S.: Biology of the human malignant lymphomas. I. Establishment in continuous cell culture and heterotransplantation of diffuse histiocytic lymphomas. Cancer 34(6): 1851-1872, Dec. 1974.

181. Epstein, M.A., Zur Hausen, H., Ball, G. and Rabin, H.: Pilot experiments with EB virus in owl monkeys (Aotus trivirgatus). III. Serological and biochemical findings in an animal with reticuloproliferative disease. Int J Cancer 15, 1975.

182. Ernberg, I., Klein, G., Kourilsky, F.M., and Silvestre, D.: Differentiation between early and late membrane antigen on human lymphoblastoid cell lines infected with Epstein-Barr virus. I. Immunofluorescence. J Natl Cancer Inst 53: 61-65, 1974.

183. Esber, H.J., Menninger, F.F., Jr. and Bogden, A.E.: Variation in serum hormone concentrations in different rat strains. Proc Soc Exp Biol Med 146: 1050-1054, Sept. 1974.

184. Evans, D.L., Barnett, J.W., and Dmochowski, L.: Immunological responsiveness in rats infected with the Soehner-Dmochowski murine sarcoma virus (MSV-SD). Tex Rep Biol Med 32(2): 449-460, 1974.

185. Evans, R., Baluda, M.A. and Shoyab, M.: Differences in the integration of avian myeloblastosis virus DNA in leukemic cells and of endogenous viral DNA in normal chicken cells. Proc Natl Acad Sci USA 71: 3152-3156, 1974.

186. Evans, R.M., Shoyab, M. and Baluda, M.A.: Studies on characterization of the integration sites of avian RNA tumor virus specific DNA. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

187. Falk, L.A., Nigida, S.M., Deinhardt, F., Wolfe, L.G., Cooper, R.W., and Hernandez-Camacho, J.I.: Herpesvirus ateles: properties of an oncogenic herpesvirus isolated from circulating lymphocytes of spider monkeys (Ateles sp.). Int J Cancer 14: 473-482, Oct. 1974.

188. Falk, L., Wright, J., Wolfe, L. and Deinhardt, F.: Herpesvirus ateles: transformation in vitro of marmoset splenic lymphocytes. Int J Cancer 14: 244-251, Aug. 1974.

189. Fenyö, E.M., Nazerian, K. and Klein, E.: Characteristics of murine C-type viruses. II. The behavior of viruses resident in various cell lines and their hybrids on BALB/3T3 and mouse embryo fibroblast cultures. Virology 59: 574-579, 1974.

190. Fidanian, H.M., Drohan, W.N. and Baluda, M.A.: The RNA of Simian sarcoma associated virus type 1 produced in human tumor cells. J Virol, March 1975.

191. Fine, D.L., Arthur, L.O.: Divalent cation requirements of RNA-dependent DNA polymerase from type B and type C virus particle. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

192. Fischinger, P. J., Blevins, C.S., and Nomura, S.: Simple quantitative assay for both xenotropic murine leukemia and ecotropic feline leukemia viruses. J Virol 14: 177-179, July 1974.

193. Fischinger, P.J. and Nomura, S.: Alteration of malignancy in cultured cells. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

194. Fleisher, T., and Kersey, J.: Immunologic mechanisms in the prevention and therapy of cancer. Am J Dis Child 128: 739-742, Nov. 1974.

195. Fleisher, T., Luckasen, J., Sabad, A., Gehrtz, R., Kersey, J.: T and B lymphocyte subpopulations in children. Pediatrics, Feb. 1975.

196. Floersheim, G.L. and Storb, R.: Knochenmarktransplantation bei aplastichen Anamien und akuten Leukamien (Bone marrow transplantation in aplastic anemias and acute leukemias). Schweiz Med Wochenschr 104: 3-10, 52-59, 87-94, 1974.

197. Forget, B.G., Marotta, C.A., Weissman, S.M., Verma, I.M., McCaffrey, R.P., and Baltimore, D.: Nucleotide sequences of human globin messenger RNA. Ann NY Acad Sci 241: 290-309, Nov. 1974.

198. Fortner, G.W., Hanna, M.G. Jr., and Coggin, J.H., Jr.: Differential effects of two strains of BCG on transplantation immunity to SV40-induced tumors in hamsters (38281). Proc Soc Exp Biol Med 147: 62-67, 1974.

199. Fraser, C.E.O. and Melendez, L.V.: Specificity differentiation (SPD) of oncogenic and non-oncogenic herpesviruses by immunofluorescence. J Med Primatol 3: 73-78, July 1974.

200. Fraser, C.E.O., Melendez, L.V. and Simeone, T.: Specificity differentiation of herpesvirus simplex types 1 and 2 by indirect immunofluorescence. J Infect Dis 130: 63-66, July 1974.

201. Friedman, M., Lilly, F., Nathenson, S.: The cell surface antigen induced by Friend murine leukemia virus is also in the virion. J Virol 14: 1126-1131, Nov. 1974.

202. Fujinaga, K., Sekikawa, K., Yamazaki, H., and Green, M.: Analysis of multiple genome fragments in adenovirus 7 transformed hamster cells. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

203. Fujinaga, K., Sekikawa, K., Yamazaki, H., and Green, M.: Multiple copies of viral genome fragments present in cells transformed by human adenoviruses. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 243-244, (abstract).

204. Fujita, D., Chen, Y., Friis, R. and Vogt, P.K.: Endogenous leukosis viruses of pheasants: In: Proc 1974 Abraham Flexner Symp on Viral Transformation and Endogenous Viruses (A. Kaplan, ed.) Academic Press, New York, 1974.

205. Fujita, D., Chen, Y., Friis, R., and Vogt, P.K.: RNA tumor viruses of pheasants. Virology 60: 558-571, 1974.

206. Gajl-Peczalska, K.J., Bloomfield, C.D., Nesbit, M.E., and Kersey, J.H.: B cell markers on lymphoblasts in acute lymphoblastic leukemia. Clin Exp Immunol 17: 561-569, Aug. 1974.

207. Gajl-Peczalska, K.J., Bloomfield, C.D., Sosin, H. and Kersey, J.H.: Value of lymphocyte study by surface markers in non-Hodgkin's malignant lymphoma. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

208. Gajl-Peczalska, K.J., Hallgren, H., Kersey, J.H., Zusman, J., and Yunis, E.J.: B lymphocytes during ageing. Lancet 2: 163, July 1974.

209. Gallagher, R.E. and Gallo, R.C.: Type C RNA tumor virus isolated from cultured human acute myelogenous leukemia cells. Science 187: 350-353, 1975.

210. Gallo, R.C., Bhattacharyya, J., Anderson, P.N.: Specific markers of chronic myelogenous leukemia (CML) cells (Phl chromosome), thymic-derived (terminal transferase and type C virus reverse transcriptase in blastic leukemia) in blastic leukemia. Fed Proc 34(3) April 1975, (abstract).

211. Gallo, R.C., Gallagher, R.E., Miller, N.R., Mondal, H., Saxinger, W.C., Mayer, R.J., Smith, R.G. and Gillespie, D.H.: Relationships between components in primate RNA tumor viruses and in the cytoplasm of human leukemic cells - Implications to leukemogenesis. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

212. Gallo, R.C., Gallagher, R., Smith, R.G. and Wu, A.: Components related to primate tumor associated type C viruses in human leukemia and in vitro approaches to inhibit their activity. Symp on Enzymes and Isozymes as Targets for Chemotherapy, Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 122 (abstract).

213. Gallo, R.C., Gallagher, R.E., Ting, R.C., Smith, R.G., Gillespie, D.H. and Wu, A.M.: Interference with formation and expression of RNA tumor virus information - Rationale and experimental results. Cancer Chemother Reports 58: 579-593, 1974.

214. Gallo, R.C., Saxinger, W.C., Gallagher, R.E., Miller, N. and Gillespie, D.: Evolutionary nature of human reverse transcriptase and of viral-related DNA synthesized in vitro by human leukemic cells. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

215. Gardner, M.B., Brown, J.C., Pike, M. and Henderson, B.E.: Feline leukemia virus: Another viewpoint. Feline Prac 4: 13-17, Nov.-Dec. 1974.

216. Gardner, M.B. and Henderson, B.E.: Lower-motor neurone disease in mice and amyotrophic lateral schlerosis in man. Lancet: 952, Oct. 1974.

217. Gardner, M.B., Officer, J.E., Parker, J., Estes, J.D. and Rongey, R.W.: Induction of disseminated virulent cytomegalovirus infection by immunosuppression of naturally chronically infected wild mice. Infect Immun 10: 966-969, Oct. 1974.

218. Gardner, M.B., Parker, J.C., Estes, J.D., and Rongey, R.W.: Induction of disseminated virulent cytomegalovirus infection by immunosuppression of naturally chronically infected wild mice. Infect Immun 10: 966-969, Oct. 1974.

219. Gardner, M.B., Rasheed, S., Rongey, R.W., Charman, H.P., Alena, B., Gilden, R.V. and Huebner, R.J.: Natural expression of feline type C virus genomes. Prevalence of detectable FeLV and RD-114 gs antigen, type C particles and infectious virus in postnatal and fetal cats. Int J Cancer 14: 97-105, July 1974.

220. Gatti, R.A., Östborn, A. and Fagraeus, A.: Selective impairment of cell antigenicity by fixation. J Immunol 113: 1361-1368, 1974.

221. Gazdar, A.F., Russell, E.K., and Minna, J.D.: Replication of mouse-tropic and xenotropic strains of murine leukemia virus in human x mouse hybrid cells. Proc Natl Acad Sci USA 71: 2642-2645, 1974.

222. Gazdar, A.F., Russell, E.K. and Minna, Z.: Replication of murine leukemia virus strains in human x mouse hybrid cells. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

223. Georgiades, J.A., Allen, P.T., Kurylo-Borowska, A. and Dmochowski, L.: Effects of edeines on cultured cells of human and animal origin. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, pp 19-20, (abstract).

224. Gerard, G., Rottman, F., and Green, M.: Poly(2'-O'methycytidylate).oligodeoxyguanylate as a template for ribonucleic acid directed deoxyribonucleic acid polymerase in ribonucleic acid tumor virus particles and a specific probe for the ribonucleic acid directed enzyme in transformed murine cells. Biochemistry 13: 1632-1641, 1974.

225. Gibson, W. and Verma, I.M.: Studies on the reverse transcriptases of RNA tumor viruses. Structural relatedness of two subunits of avian RNA tumor viruses. Proc Natl Acad Sci USA 71: 4991-4994, 1974.

226. Gilden, R.V.: Interrelationships among RNA tumor viruses and host cells. In: Advances in Cancer Research (G. Klein and S. Weinhouse, eds.) Academic Press, New York, N.Y.

227. Gillette, R.W.: Homing properties of transplantable lymphoma cell lines. J Reticuloendothel Soc 16: 46a, 1974.

228. Gillette, R.W. and Boone, C.W.: Effect of tumor immunity on the distribution of labeled lymphoid cells in tumor-challenged mice. Cell Immunol 14: 386-393, 1974.

229. Gillette, R.W., McKenzie, G.O. and Swanson, M.H.: Modification of the lymphocyte response to mitogens by cyclic AMP and GMP. J Reticulondothel Soc 16: 289-299, 1974.

230. Gisselbrecht, S., Bassin, R.H., Gerwin, B.I. and Rein, A.: Dual susceptibility of a 3T3 mouse cell line to infection by N- and B-tropic murine leukemia virus: Apparent lack of expression of the Fv-1 gene. Int J Cancer 14: 106-113, July 15, 1974.

231. Glaser, R., Decker, B., Ladda, R. and Shows, T.: Characteristics of cellular transformation associated with the Epstein-Barr virus in non-lymphoblastoid cells: Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11. 1975. San Diego, California, (abstract).

232. Glaser, R. and Farrugia, R.: Effect of the host cell on the replication of the Epstein-Barr virus. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

233. Glaser, R. and Nonoyama, M.: Host cell regulation of induction of Epstein-Barr virus. J Virol 14(1): 174-176, July 1974.

234. Glaser, R. and Rapp, F.: Control of the Epstein-Barr virus genome by mammalian cells. Symp on Fundamental Aspects of Neoplasia, 1974, p 33, (abstract).

235. Glucksberg, H., Storb, R., Fefer, A., Buckner, C.D., Neiman, P.E., Clift, R.A., Lerner, K.G. and Thomas, E.D.: Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A matched sibling donors. Transplantation 18: 295-304, 1974.

236. Goldberg, R.J., Scolnick, E.M. and Parks, W.P.: Isolation of a primate type C virus from a lymphomatous baboon. Int J Cancer 14: 722-730, 1974.

237. Goodheart, C.R. and Miyagi, M.: Isolation and characterization of the DNA of herpesvirus saimiri. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

238. Grandgenett, D.P. and Green, M.: Different mode of action of ribonuclease H in purified α and αβ ribonucleic acid-directed deoxyribonucleic acid polymerase from avian myeloblastosis virus. J Biol Chem 249(16): 5148-5152, 1974.

239. Grant, J.P., Bigner, D.D., Fischinger, P.J. and Bolognesi, D.P.: Expression of murine leukemia virus structural antigens on the surface of chemically induced murine sarcomas. Proc Natl Acad Sci USA 71(12): 5037-5041, Dec. 1974.

240. Green, M. and Gerard, G.F.: RNA-directed DNA polymerase - properties and functions in oncogenic RNA viruses and cells. In: Progress in Nucleic Acid Research and Molecular Biology, Vol 14, Academic Press, New York, 1974, pp 187-334.

241. Green, M., Gerard, G.F., Grandgenett, D.P., Gurgo, C., Rankin, A.M., Green, M.R. and Cassell, D.M.: Biochemical suppression and tumor virus activity. Cancer 34(4): 1427-1438, 1974.

242. Green, M., Grandgenett, D., Gerard, G., Rho, H.M., Loni, M.C., Robins, M., Salzberg, S., Shanmugam, G., Bhaduri, S. and Veccio, G.: Properties of oncornavirus RNA-directed DNA polymerase, the RNA template, and the intracellular products formed early during infection and cell transformation. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

243. Green, M., Yamashita, T., Brackmann, K., Fujinaga, K., Arens, M., Buettner, W., Shamugam, G. and Bhaduri, S.: Adenovirus DNA: Transcription during productive infection, integration in transformed cells, and replication in vitro. In: Viral Transformation and Endogenous Viruses. (A. Kaplan, ed.) Academic Press, New York, 1974, pp 19-34.

244. Greenberger, J.S. and Aaronson, S.A.: Cycloheximide induction of xenotropic type C virus from synchronized mouse cells: Metabolic requirements for virus activation. J Virol 15: 64-70, Jan. 1975.

245. Greenberger, J.S., Anderson, G.R. and Aaronson, S.A.: Transformation-defective virus mutants in a class of morphologic revertants of sarcoma virus-transformed nonproducer cells. Cell 2: 279-286, Aug. 1974.

246. Greenberger, J.S., Stephenson, J.R., Aoki, T. and Aaronson, S.A.: Cell surface antigens of murine sarcoma virus transformed nonproducer cells: Further evidence for lack of transplantation immunity. Int J Cancer 14: 145-152, Sept. 1974.

247. Greenberger, J.S., Stephenson, J.R., Moloney, W.C. and Aaronson, S.A.: Different hematologic diseases induced by type C viruses chemically activated from embryo cells of different mouse strains. Cancer Res 35: 245-252, Jan. 1975.

248. Guerra, A., Koldovsky, U. and Henle, W.: Antibodies unrelated to Epstein-Barr virus in human sera which precipitate soluble components of cultured lymphoblasts. J Natl Cancer Inst 53: 1193-1200, 1974.

249. Gunvén, P.: Clinical correlations to Epstein-Barr virus associated antibody titers in Burkitt's lymphoma. Nord Cancerunions Symp Tumorimmunol Diagnosis, Stockholm, 1974, (abstract).

250. Gunvén, P.: The clinical significance of immunological findings in Burkitt's lymphoma. (Epstein-Barr virus-associated antibodies/BCG treatment). In: Modern Trends in Human Leukemia. (R. Neth, R.C. Gallo, S. Spiegelman and F. Stohlman, Jr., eds.) J.F. Lehmanns Verlag, München, 1974, pp 129-132.

251. Gunvén, P.: Epstein-Barr virus-associated antibodies in Burkitt's lymphoma in relation to the clinical course of the disease. Academic Thesis, Stockholm, 1974.

252. Gunvén, P., Klein, G., Clifford, P. and Singh, S.: Epstein-Barr virus-associated membrane-reactive antibodies during long term survival after Burkitt's lymphoma. Proc Natl Acad Sci USA 71: 1422-1426, 1974.

253. Gupta, P., Johnson, J.C. and August, J.T.: Synthesis of the structural proteins of Rous sarcoma virus. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

254. Hackett, A.J., Springer, E.L. and Byers, V.: The surface topology of human peripheral blood lymphocytes. XIVth Annu Mtg Am Soc Cell Biol, San Diego, California 63(2): 253, Nov. 1974, (abstract).

255. Hadidi, A. and Fraenkel-Conrat, H.: Host range and structural data on common plant viruses. In: Handbook of Genetics. Vol II. (R.C. King, ed.) Plenum Publishing Co., New York, New York, 1974, pp 382-413.

256. Halpern, M.S., Bolognesi, D.P. and Lewandowski, L.J.: Isolation of the major viral glycoprotein and a putative precursor from cells transformed by avian sarcoma viruses. Proc Natl Acad Sci USA 71(6): 2342-2364, 1974.

257. Halpern, M.S., Hunter, E., Alevy, M., Friis, R.R. and Vogt, P.K.: Immunological studies with avian RNA tumor viruses. In: Advances in the Biosciences 12 (G. Raspe and S. Bernhard, eds.) Pergamon Press-Vieweg, 1974, pp 567-577.

258. Hampar, B., Derge, J.G., Nonoyama, M., Chang, S., Tagamets, M.A. and Showalter, S.D.: Programming of events in Epstein-Barr virus-activated cells induced by 5-iododeoxyuridine. Virology 62(1): 71-89, Nov. 1974.

259. Hampar, B., Derge, J.G., Tanaka, A. and Nonoyama, M.: Sequence of Epstein-Barr virus productive cycle in human lymphoblastoid cells. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

260. Hanna, M.G., Jr., Ihle, J.N., Batzing, B.L., Tennant, R.W. and Schenley, C.L.: Assessment of reactivities of natural antibodies to endogenous RNA tumor virus envelope antigens and virus-induced cell surface antigens. Cancer Res 35: 164-171, 1975.

261. Hannon, W.H., Anderson, N.G. and Coggin, J.H., Jr.: Relationship of sialic acid to the expression of fetal antigens in the developing hamster fetus. In: Chemical Carcinogenesis Part B (P.O. Tso and J.A. DiPaolo, eds.) Marcell Dekker, Inc., 1974, pp 669-682.

262. Haspel, M.V. and Rapp, F.: Genetic analysis of the encephalitogenic potential of measles virus. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

263. Haspel, M.V. and Rapp, F.: Measles virus: An unwanted variant causing hydrocephalus. Science 187: 450-451, 1975.

264. Hay, K. and Lausch, R.: Virus specific membrane antigen(s) common to cells infected or transformed by herpes simplex virus type 1. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

265. Hayflick, L.: The choice of a cell substrate for preparation of human interferon. In Vitro, Monograph No. 3: 4-11, 1974.

266. Heberling, R.L. and Kalter, S.S.: Use of nonhuman primates in virus research. Lab Anim Sci 24(1): 142-149, 1974.

267. Heberling, R.L., Kalter, S.S., Barker, S.T., Smith, G.C., and Hellman, A.: Infection of beagles with an endogenous baboon C-type virus. Fed Proc 34(3) April 1975, (abstract).

268. Heiniger, H.J., Taylor, B.A., Hards, E.J. and Meier, H.: Heritability of the PHA responsiveness of lymphocytes and its relationship to leukemogenesis. Cancer Res 35: 857-863, 1975.

269. Hellman, A., Peebles, P.T., Strickland, J.E., Fowler, A.K., Kalter, S.S., Oroszlan, S. and Gilden, R.V.: Baboon virus isolate M-7 with properties similar to feline virus RD-114. J Virol 14(1): 133-138, July 1974.

270. Hellström, I. and Hellström, K.E.: Cell-mediated immune reactions to tumor antigens with particular emphasis on immunity to human neoplasms. Cancer 34: 1461-1468, 1974.

271. Hellström, I. and Hellström, K.E.: Cytotoxic effect of lymphocytes from multiparous mice on cultivated tumor cells. II. Evidence that "embryonic" tumor antigens elicit high titered blocking serum factors, that mice with transplantation resistance to chemically induced sarcomas often have blocking factors to embryonic antigens, and that "unblocking" antisera to such antigens may be raised in rabbits. Int J Cancer 15, Jan. 1975.

272. Hellström, I. and Hellström, K.E.: Cytotoxic effect of lymphocytes from pregnant mice on cultivated tumor cells. I. Specificity, nature of effector cells, blocking by serum. Int J Cancer 15, Jan. 1975.

273. Hellström, K.E., and Hellström, I.: Critical factors in cancer immunology. 7th Annual Miami Winter Symp, Vols 9 & 10, Miami, Fla., Jan. 1975, (abstract).

274. Hellström, K.E. and Hellström, I.: Lymphocyte mediated cytotoxicity and blocking serum activity to tumor antigens. In: Advances in Immunology, 18 (F.J. Dixon, ed.), 1974, pp 209-277.

275. Henderson, B.E.: Type B virus and human breast cancer. Cancer 34: 1386-1389, Oct. 1974.

276. Henderson, B.E., Gardner, M.B., Gilden, R.V., Estes, J.D. and Huebner, R.J.: Prevention of lower limb paralysis by neutralization of type C RNA virus of wild mice. J Natl Cancer Inst 53: 1091-1092, Oct. 1974.

277. Henderson, B.E., Powell, D., Rosario, I., Keys, C., Hanisch, R., Young, M., Casagrande, J., Gerkins, V. and Pike, M.C.: An epidemiologic study of breast cancer. J Natl Cancer Inst 53: 609-614, Sept. 1974.

278. Henderson, I.C., Lieber, M.M. and Todaro, G.J.: Mink cell line MvlLu: Focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology 60: 282-287, July 1974.

279. Henle, G., Henle, W. and Horwitz, C.A.: Antibodies to Epstein-Barr-virus-associated nuclear antigen in infectious mononucleosis. J Infect Dis 130: 231-239, 1974.

280. Henle, W. and Henle, G.: Epstein-Barr and human malignancies. Cancer 34: 1368-1374, 1974.

281. Henle, W. and Henle, G.: The Epstein-Barr virus (EBV) in Burkitt's lymphoma and naso-pharyngeal carcinoma. Ann Clin Lab Sci 4: 109-114, 1974.

282. Henle, W., Henle, G., Hewetson, J.F., Rocchi, G., and Leikola, J.: Failure to detect heterophile antigens in EBV-infected cells and to demonstrate interaction of hetero-phile antibodies with EBV. Clin and Exp Immunol 17: 281-286, 1974.

283. Henle, W., Henle, G. and Horwitz, C.A.: Epstein-Barr virus-specific diagnostic tests in infectious mononucleosis. Hum Pathol 5: 551-556, 1974.

284. Herberman, R.B., Aoki, T., Nunn, M., Lavrin, D.H., Soares, N., Gazdar, A., Holder, H., and Chang, K.S.S.: Specificity of ^{51}Cr-release cytotoxicity by lymphocytes immune to murine sarcoma virus. J Natl Cancer Inst 53: 1103-1111, Oct. 1974.

285. Herberman, R.B., Char, D., Oldham, R., Levine, P., Leventhal, B.G., McCoy, J.L., Ho, J.C., and Chau, J.C.W.: Cell-mediated immunity in human acute leukemia. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

286. Hersh, E.M., Gutterman, J.U., Mavligit, G., Gschwind, C.R., Freireich, E.J., Levine, P.H. and Plata, E.J.: Human immune response to active immunization with Rauscher leukemia virus. 1. Cell-mediated and cell-associated immunity. J Natl Cancer Inst 53(2): 317-325, Aug. 1974.

287. Hersh, E.M., Gutterman, J.U., Mavligit, G.M., Gschwind, C.R., and Hanna, M.G., Jr.: Human immune response to Rauscher leukemia virus. Haematol Bluttransfus 14: 119-128, 1974.

288. Hersh, E.M., Hanna, M.G., Jr., Gutterman, J.U., Mavligit, G., Yurconic, M., Jr. and Gschwind, C.R.: Human immune response to active immunization with Rauscher leukemia virus. II. Humoral immunity. J Natl Cancer Inst 53(2): 327, Aug. 1974.

289. Hilgers, J., Decléve, A., Galesloot, J. and Kaplan, H.S.: Murine leukemia group-specific antigen expression in AKR mice. Cancer Res 34(10): 2553-2561, Oct. 1974.

290. Hilgers, J., and Lamie, F.: Genetic aspects of susceptibility to leukemia in mice. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, p 125, (abstract).

291. Hilgers, J., Tulp, A., Bont, W., Calafat, J., Crusius, B., Uitendaal, van Blitterswijk, W., Souissi, T., Haverman, J., Nusse, R. and Verbeet, M.: Expression of mammary tumor virus antigens as a function of the life cycle. In: Proc IX Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

292. Hilgers, J., van Blitterswijk, W., Bont, W., Nusse, R., Haverman, J., Theuns, G., Feltkamp, C., Calafat, J., van Nie, R., Hageman, Ph. and Emmelot, P.: Biochemical and physical characteristics of a mammary tumor virus induced cell surface antigen as compared with a normal antigen. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

293. Hilleman, M.R.: Human cancer virus vaccines and the pursuit of the practical. Cancer 34: 1439-1445, 1974, (abstract).

294. Hilleman, M.R.: Immunity to cancer. Naturally occurring tumors in domestic animals as models for research: Part I and Part II. Bull WHO 49: 81-89; 205-213, 1973, (abstract).

295. Hiraki, S., Chan, J.C., Hales, R.L., and Dmochowski, L.: An immunoelectron microscopy study of Soehner-Dmochowski murine sarcoma virus following passages in rats and hamsters. Cancer Res 34(11):2906-2910, Nov. 1974.

296. Hirsch, M.S. and Black, P.H.: Activation of mammalian leukemia viruses. In: Advances in Virus Research Vol 19, Academic Press, 1974, pp 265-313.

297. Hirsch, M.S., Proffitt, M.R., Black, P.H. and Ellis, D.A.: Autoaggressive reactions and leukemia virus infections: Interrelated events in their pathogenesis. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 279-287.

298. Holland, J.F., Roboz, J. and Bekesi, J.G.: Antineoplastic effects of Virazole on AKR leukemia. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

299. Holland, J.F., Wald, N., Roboz, J.P. and Bekesi, J.G.: Combined anti-viral chemoimmunotherapy in leukemic AKR mice. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 365, (abstract).

300. Hollinshead, A.C., Tarro, G., Chretien, P. and Rawls, W.: Characterization of Herpesvirus nonvirion antigens: Relation to squamous cell carcinomas. In: Viral Immunodiagnosis (E. Kurstak and R. Morisset, eds.) Academic Press, New York 1974, pp 301-317.

301. Hollis, V.W., Jr., Aoki, T., and Barrera, O.L.: Naturally occurring antibodies to RNA-dependent DNA polymerase of mouse type B and type C RNA tumor viruses in kidney eluates of C3H/He mice. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

302. Hoover, E.A. and Kociba, G.J.: Bone lesions in cats with anemia induced by feline leukemia virus. J Natl Cancer Inst 53: 1277-1284, 1974.

303. Hoover, E.A., Kociba, G.J., Hardy, W.D., Jr., and Yohn, D.S.: Erythroid hypoplasia in cats inoculated with feline leukemia virus. J Natl Cancer Inst 53: 1271-1276, 1974.

304. Howk, R.S., Williams, D.R., Haberman, A.B., Parks, W.P. and Scolnick, E.M.: A comparison of the transcription of mouse cell chromatin by homologous RNA polymerase I and II. Cell 3: 15-22, 1974.

305. Huang, D.P., Ho, J.H.C., Henle, W., and Henle, G.: Demonstration of EBV-associated nuclear antigen in NPC cells from fresh biopsies. Intl J Cancer 14: 580-588, 1974.

306. Hunter, E., Friis, R.R. and Vogt, P.K.: Inhibition of avian sarcoma virus replication by glucosamine. Virology 58: 449-456, 1974.

307. Ihle, J.N., Hanna, M.G., Jr., Schäfer, W., Hunsmann, G., Bolognesi, D.P. and Hüper, G.: Polypeptides of mammalian oncornaviruses. III. Localization of p15 and reactivity with natural antibody. Virology 63: 60-67, 1975.

308. Ihle, J.N., Kenney, F.T. and Tennant, R.W.: Evidence for a stable intermediate in leukemia virus activation in AKR mouse embryo cells. J Virol 14(3): 451-456, Sept. 1974.

309. Ihle, J.N., Lane, S.E., Kenney, F.T., and Farrelly, J.G.: Effect of glucocorticoids on activation of leukemia virus in AKR mouse embryo cells. Cancer Res 35: 442-446, Feb. 1975.

310. Ikeda, H., Pincus, T., Yoshiki, T., Strand, M., August, J.T., Boyse, E.A., and Mellors, R.C.: Biological expression of antigenic determinants of murine leukemia virus proteins gp69/71 and p30. J Virol 14: 1274-1280, 1974.

311. Ishimoto, A., Suzuki, Y., Yoshida, T. and Ito, Y.: Further studies on mouse fetal antigen cross-reactive with Rauscher leukemia. Cancer Res 34, 2338-2343, 1974.

312. Ishimoto, A., Suzuki, Y., Yoshida, T., and Ito, Y.: Mouse fetal antigens in Rauscher leukemia. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 311-317.

313. Jenson, A.B., Groff, D.E., Byers, M.J., and McConahey, P.J.: Vertical transmission of MuLV (Scripps) in mice. Fed Proc 34(3) April 1975, (abstract).

314. Johnson, F.L., Thomas, E.D., Buckner, C.D., Clift, R.A., Fefer, A. and Storb, R.: The current status of bone marrow transplantation in cancer treatment. Cancer Treatment Rev I: 81-97, 1974.

315. Johnson, J.C., Higuchi, T., and Geissler, E.: Isolation and antigenic analysis of woolly monkey RNA tumor virus structural proteins. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

316. Johnson, R.T., Narayan, O. and Weiner, L.P.: The relationship of SV40 related viruses to progressive multifocal leukoencephalopathy. In: Mechanisms of Virus Disease (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 187-197.

317. Joseph, B.S. and Oldstone, M.B.A.: Expression of selected antigens on the surface of cultured neural cells. Brain Res 80: 421-434, 1974.

318. Kalter, S.S.: Significance of simian viruses. In: Animals in Medicine. (L.T. Hermison, ed.) Government Printing Office, Washington, D.C., 1974, pp 965-975.

319. Kalter, S.S. and Heberling, R.L.: Biohazards and simian viruses. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 655-665.

320. Kalter, S.S., Heberling, R.L., Helmke, R.J., Panigel, M., Smith, G.C., Kramer, D.C., Hellman, A., Fowler, A.K. and Strickland, J.E.: Comparative studies on the presence of C-type viral particles in placentas from primates and other animals. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

321. Kalter, S.S., Kuntz, R.E., Heberling, R.L., Helmke, R.J. and Smith, G.C.: C-type particles in a urinary bladder neoplasm induced by Schistosoma haematobium. Nature 251(5474): 440, 1974.

322. Kaplan, J., Cejka, J. and Peterson, W.D., Jr.: Malignant T lymphoblasts bind human immunoglobulin. Pediatr Res 8: 140, 1974.

323. Kaplan, J., Mastrangelo, R., Peterson, W.D., Jr.: Identification of tumor antigens on lymphoblasts and lymphoid cell lines from children with thymic lymphoblastic lymphoma. Proc IVth Mtg Eur Soc Pediatr Haematol and Immunol, 1974.

324. Kaplan, J., Shope, T.C. and Peterson, W.D., Jr.: Characterization of established T and B cell lines in vitro. In Vitro: 9, 375, 1974, (abstract).

325. Kaplan, J., Shope, T.C. and Peterson, W.D., Jr.: Epstein-Barr virus negative human malignant T-cell lines. J Exp Med 139: 1070, 1974.

326. Kaplan, J., Tilton, J. and Peterson, W.D., Jr.: Identification of T cell lymphoma tumor antigens on human T cell lines. Fed Proc 34(3) April 1975, (abstract).

327. Kaplan, J., Tilton, J. and Peterson, W.D., Jr.: T cell lymphoma antigen of human T cell lines. In Vitro, 1975, (abstract).

328. Kawakami, T.G., Buckley, P.M., and DePaoli, A.: Gibbon antibody response to gibbon type C virus infection. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

329. Kawakami, T.G., Buckley, P.M., and DePaoli, A.: Type C virus associated experimental granulocytic leukemia in a primate. Am J Clin Pathol Oct. 1974, (abstract).

330. Kawakami, T.G., Buckley, P.M., DePaoli, A., Noll, W. and Bustad, L.K.: Studies on prevalence of type C virus associated with gibbon hematopoietic neoplasms. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

331. Kay, H.D. and Sinkovics, J.G.: Cytotoxic lymphocytes from normal donors. Lancet 2: 296-297, 1974.

332. Kay, H.D., Thota, H., Romero, J.J., Sinkovics, J.G.: Lymphocytes from normal donors are frequently cytotoxic to tumor cells. Fed Proc 34(3) April 1975, (abstract).

333. Kersey, J., Nesbit, M., Luckasen, J., Hallgren, H., Sabad, A., Yunis, E., and Gajl-Peczalska, K.: Acute lymphoblastic leukemic and lymphoma cells with thymus-derived (T) markers. Mayo Clinic Proc 49: 584-587, Aug. 1974.

334. Kersey, J., Spector, B., and Good, R.A.: Primary immunodeficiency and malignancy. In: Immunodeficiency in Man and Animals. (D. Bergsma, ed.) Birth Defects: Vol XI(1), Sinauer Press, 1975.

335. Kessler, I.I. and Aurelian, L.: Uterine cervic cancer. In: Cancer, Epidemiology and Prevention: Current Concepts, (D. Schottenfeld, ed.) Thomas Co., Springfield, Jan. 1975.

336. Keydar, J., Graslawsky, G., Dajbog, T., Karby, S., Shinedling, S.T., Weiss, F. and Delarea, J.: Brief Communication: Intramitochondrial bodies in a human cell line carrying oncornavirus-like particles. J Natl Cancer Inst 54, Jan. 1975.

337. Keyder, J., Braslawsky, G., Dajbog, T., Karby, S., Shinedling, S.T., Weiss, F., and Delarea, J.: Intramitochondrial bodies in a human cell line carrying oncornavirus-like particles. In: Proc IX Mtg on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

338. Kingsbury, D.T. and Lerner, R.A.: Encapsulation of lymphocyte DNA by vesicular stomatitis virus. Proc Nat Acad Sci USA 71: 1753-1757, 1974.

339. Kithier, K., Cejka, J., Belamaric, J., Al-Sarraf, M., Peterson, W.D., Jr., Vaitkevicius, V.K., Poulik, M.D.: Beta2-Microglobulin: Occurrence in fetal life and malignancy. Clin Chim Acta 52: 293, 1974.

340. Klein, D.L., Anderson, P.N., Santos, G.W.: Lymphocyte cytotoxicity to leukemic blast cells by normal individuals. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

341. Klein, G.: Epstein-Barr virus (EBV). Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

342. Klein, G.: Studies on the Epstein-Barr virus (EBV)-genome and the EBV-determined nuclear antigen in human malignant disease. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

343. Klein, G., Lindahl, T., Jondal, M., Leibold, W., Menezes, J., Nilsson, K., and Sundström, C.: Continuous lymphoid cell lines with characteristics of B cells (bone-marrow-derived), lacking the Epstein-Barr virus genome and derived from three human lymphomas. Proc Natl Acad Sci USA 71: 32B3-3286, 1974.

344. Klein, G., Steiner, M., Wiener, F., and Klein, E.: Human leukemia-associated anti-nuclear reactivity. Proc Natl Acad Sci USA 71: 685-689, 1974.

345. Knight, P., Duff, R., Glaser, R. and Rapp, F.: Characteristics of the release of measles virus from latently infected cells after co-cultivation with BSC-1 cells. Intervirology, 2: 287-298, 1974.

346. Kniker, W.T., Macias, E.G., Steele, R.W., Heberling, R.L., and Eller, J.J.: Relative cellular immunity in three nonhuman primate species. Fed Proc 34(3) April 1975, (abstract).

347. Kolb, H.J., Storb, R., Weiden, P.L., Ochs, H.D., Kolb, H., Graham, T.C., Floersheim, G.L. and Thomas, E.D.: Immunologic, toxicologic and marrow transplantation studies in dogs given dimethyl myleran. Biomedicine 20: 341-351, 1974.

348. Kotler, M., Haspel, O. and Becker, Y.: Synthesis of double-stranded DNA molecules by a cytoplasmic RNase sensitive DNA polymerase activity from Rous sarcoma virus transformed rat cells. Nature 249: 441-444, 1974.

349. Kouri, R.E., Kiefer, R. and Zimmerman, E.M.: Hydrocarbon metabolizing activity of various mammalian cells in culture. In Vitro 10: 18-25, 1974.

350. Kudo, T. and Aoki, T.: Specific antigens on the surface of tumor cells. In: Tumor Immunology (H. Kobayashi and T. Tachibana, eds.) Asakura Publishing Company, 1974, pp 37-45 (In Japanese).

351. Kung, H., Bailey, J.M., Davidson, N., Nicolson, M.O. and McAllister, R.M.: Structure and molecular length of the large subunits of RD-114 viral RNA. J Virol 14: 170-173, 1974.

352. Kung, H., Bailey, J.M., Davidson, N., Vogt, P., Nicolson, M.O. and McAllister, R.M.: Electron microscope studies of tumor virus RNA. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

353. Kuo, E.Y.H., Cobb, W.R., Esber, H.J. and Bogden, A.E.: Effects of hysterectomy on milk secretion and serum levels of prolactin, growth hormone, estrogen and progesterone in Rhesus monkeys with hormone-induced uterine hypertrophy. Am J Obstet Gynecol 120: 368-375, Oct. 1974.

354. Kuo, E.Y.H., Esber, H.J. and Bogden, A.E.: Radioimmunoassay for Mason-Pfizer monkey virus Gs-1 antigen. Fed Proc 34(3) April 1975, (abstract).

355. Lamon, E.W., Hilgers, F., Rosen, A. and Klein, G.: Radioiodine-labeled antibody elution (RIE) for detection of EBV-determined antigens: Competition for specific labeled antibodies using suspended antigens. Clin Immunol Immunopathol 2: 542-555, 1974.

356. Lamon, E.W., Whitten, H.D., Skurzak, H.M., Andersson, B. and Klein, E.: Tumor cell destruction by lymphocytes induced with tumor specific IgM. 7th Annual Miami Winter Symp, Vols 9 & 10, Miami, Fla., Jan. 1975, (abstract).

357. Lapis, K., Beard, D. and Beard, J.W.: Transplantation of hepatomas induced in the avian liver by MC29 leukosis virus. Cancer Res 35(1): 132-138, Jan. 1975.

358. Larsen, C.J., Emanoil-Ravicovitch, R., Hampe, A., Mauchauffé, M., Bazilier, M. and Robert-Robin, J.: Identity of the two 8S component of the mouse sarcoma virus (Moloney). Nucleic Acid Res 1: 849-854, 1974.

359. Larsen, C.J., Marty, M., Tavitian, A., Hamelin, R., Périés, J. and Boiron, M.: Homology between genome of a murine oncornavirus and RNA rich in polyadenylate of human leukemia cells. Comptes Rendus Acad Sci (série D), 279(7): 619-622, 1974.

360. Larsen, C.J. and Tavitian, A.: Données actuelles sur l'état intégré des virus leucémogènes et sarcomatogènes. Actual Hématolog (Masson et Cie, eds.) Paris, 1974, pp 271-277.

361. Lasfargues, E.Y. and Moore, D.H.: Search for a viral etiology of human breast cancer. J Invest Dermatol 63: 149-159, July 1974.

362. Lasfargues, E.Y., Vaidya, A.B. and Coutinho, W.G.: New observations on the possible viral etiology of human breast carcinomas. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

363. Leavitt, R.W., Sparks, R.B., Jr., Helinski, D.R. and Lerner, R.A.: Immunoelectron microscopic localization of single-stranded regions in DNA. Biochem/Biophys Mtg, 1974.

364. Lee, J.C., Hanna, M.G., Jr., Ihle, J.N. and Aaronson, S.A.: Autogenous immunity to endogenous RNA tumor virus: Differential reactivities of immunoglobulins M and G to virus envelope antigens. J Virol 14(4): 773-781, Oct. 1974.

365. Lee, K.L. and Nickol, J.M.: Phosphorylation of tyrosine aminotransferase in vivo. J Biol Chem 249(18): 6024-6026, Sept. 1974.

366. Leighton, P., Smith, P.G., Draper, G.J. and Pike, M.C.: Malignant disease in the parents of children dying of Hodgkin's disease. Br J Cancer 30: 373-375, Oct. 1974.

367. Lerner, R.A., Dixon, F.J., Croker, B.P., Del Villano, B.C., Jensen, F.C., Kennel, S.J. and McConahey, P.J.: The possible role of oncornaviruses in the etiology and pathogenesis of murine lupus. In: Adv in Biosciences, Vol 12, (G. Raspe, ed.), Pergamon Press/Vieweg, 1974, pp 356-365.

368. Levin, A.C., Massey, R.J. and Deinhardt, F.: Inhibition of lymphocytotoxicity in human breast cancer. The role of lymphocyte preparation. IXth Leukocyte Culture Conference, 1974, p 50, #129, (abstract).

369. Levin, A.C., Massey, R.J., Deinhardt, F., Schauf, V. and Wolter, J.: The effect of long term chemotherapy on lymphocytotoxicity in human breast cancer. IXth Leukocyte Culture Conference, 1974, p 49, #128, (abstract).

370. Levine, P.H., Diamond, R.D., Reisher, J.I.: Antibodies to Epstein-Barr virus (EBV) in patients with cryptococcosis. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

371. Levine, P.H. and Gravell, M.: The viral etiology of leukemia. In: Modern Problems in Paediatrics, Vol 16 (W. Schuler, ed.) S. Karger, Basel, 1974, pp 136-164.

372. Lilly, F., Duran-Reynals, M. and Rowe, W.: Murine leukemia virus and spontaneous leukemia in AKR mice. In: Mechanisms of Virus Disease, Vol I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 41-47.

373. Lilly, F. and Steeves, R.: Antigens of murine leukemia viruses. Biochim Biophys Acta-Reviews on Cancer 1: 105-118, 1975.

374. Long, J.C., Aisenberg, A.C. and Zamecnik, P.C.: An antigen in Hodgkin's disease tissue cultures: Radioiodine-labeled antibody studies. Proc Natl Acad Sci USA 71: 2605-2609, 1974.

375. Loni, M.C. and Green, M.: Detection and localization of virus-specific DNA by in situ hybridization of cells during infection and rapid transformation by the murine sarcoma-leukemia virus. Proc Natl Acad Sci USA 71(9): 3418-3422, 1974.

376. Loni, M.C. and Green, M.: Virus-specific DNA sequences in mouse and rat cells transformed by the Harvey and Moloney murine sarcoma viruses detected by in situ hybridization. Virology 63: 40-47, 1975.

377. Lopez, D.M., Ortiz-Muniz, G. and Sigel, M.M.: Response of lymphocytes from MTV-free mice to MTV-associated antigens. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

378. Lopez, D.M., Sigel, M.M. and Meyers, P.: Correlation of in vitro lymphocyte response to mammary tumor antigens with in vivo immunogenicity. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

379. Lovinger, G.G., Ling, H.P., Klein, R.A., Gilden, R.V. and Hatanaka, M.: Unintegrated murine leukemia viral DNA in newly infected cells. Virology 62: 280-283, Nov. 1974.

380. Lowy, D.R., Chattopadhyay, S.K., Teich, N.M., Rowe, W.P. and Levine, A.S.: AKR murine leukemia virus genome: frequency of sequences in DNA of high-, low-, and non-virus-yielding mouse strains. Proc Natl Acad Sci USA 71: 3555-3559, Sept. 1974.

381. Luckasen, J., Sabad, A., Goltz, R., and Kersey, J.: T and B lymphocytes in atopic eczema. Arch Dermatol 110, 375-377, Sept. 1974.

382. Luckasen, J., White, J., and Kersey, J.: Mitogenic properties of a calcium ionophore, A23187. Proc Natl Acad Sci USA 71: 5088-5090, Dec. 1974.

383. Luftig, R.B., McMillan, P.N. and Bolognesi, D.P.: An ultrastructural study of C-type virion assembly in mouse cells. Cancer Res 34: 3303-3310, 1974.

384. Luftig, R.B., McMillan, P.N., Culbreth, K. and Bolognesi, D.P.: A determination of the outer dimensions of oncornaviruses by several electron microscopic procedures. Cancer Res 34: 1694-1706, 1974.

385. Lynch, H.T., Guirgis, H., Albert, S. and Brennan, M.: Familial breast cancer in a normal population. Cancer 34(6): 2080-2086, Dec. 1974.

386. Macario, A.J.L., and Conway de Macario, E.: Association constant and heterogeneity index of antibodies specific for a natural determinant of a bacterial protein. Time-course studies during the in vitro immune response to a distinct site of E. coli -D-galactosidase. J Immunol 113, 756, 1974.

387. Macario, A.J.L., and Conway de Macario, E.: Synthesis of low affinity antibody molecules during the decline of the immune response. Immunochemistry 11: 619-621, 1974.

388. Madahar, D.P., Dosik, H., and Wexler, I.: E 18 XXX-double trisomy: A case report. J Med Genetics 11: 309-311, 1974.

389. Major, E.O. and Di Mayorca, G.: In vitro transformation of primate cells by a rodent papovavirus (polyoma) and a human papovavirus (BK). Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

390. Mangel, W.F., Delius, H. and Duesberg, P.H.: Structure and molecular weight of the 60-70S and the 30-40S RNA of the Rous sarcoma virus. Proc Natl Acad Sci USA 71(11): 4541-4545, Nov. 1974.

391. Mangel, W.F., Delius, H. and Duesberg, P.H.: The structure and molecular weight of the 60-70S RNA and the 30-40S RNA of the Rous sarcoma virus. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

392. Martin, W.J.: Immune surveillance directed against derepressed cellular and viral alloantigens. Cell Immunol 15:1-10, 1975.

393. Marty, M., Mauchauffé, M., Poli, J. and Larsen, C.J.: Isolement des acides ribonucléiques riches en acide polyadénylique par chromatographie sur colonne de cellulose non esterifiée. Biochimie 56: 659-665, 1974.

394. Maruyama, K.: Biological aspects of transformation of human cells by viruses. Bull Cancer 26 (4): 65-68, July-Aug., 1974.

395. Maruyama, K. and Dmochowski, L.: Antigenic analysis of transformed and nontransformed cells from human neoplasms. In: Immunology of Cancer. Progress in Experimental Tumor Research, Vol 19, Karger, Basel, 1974, pp 253-269.

396. Maruyama, K. and Dmochowski, L.: A search for transforming agents in human tumors. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 296-297, (abstract).

397. Maruyama, K., East, J.L., Wagner, S.H., and Dmochowski, L.: In vitro transformation of cells from human neoplasms. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

398. Maruyama, K., Miller, M.F., Wagner, S., and Dmochowski, L.: An immunoelectron microscopy study of type C virus particles in feline sarcoma virus-induced rat tumors. Proc 32nd Annual Mtg Electron Microscopy Society of America, Aug. 1974, pp 246-247, (abstract).

399. Maruyama, K., Wagner, S.H. and Dmochowski, L.: Sarcomas induced in rats by feline RNA virus. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

400. Mason, W.S., Friis, R.R., Linial, M. and Vogt, P.K.: Determination of the defective function in two mutants of Rous sarcoma virus. Virology 61: 559-574, 1974.

401. Massey, R., Falk, L., Paciga, J. and Deinhardt, F.: T and B cells in cell-mediated lympholysis of autologous cells by sensitized and nonsensitized lymphocytes. IXth Leukocyte Culture Conference, 1974, p 53, #142, (abstract).

402. Massey, R.J., Levin, A.C. and Deinhardt, F.: Antibody-dependent lymphocytotoxicity (ADL) and blocking of ADL in human breast cancer. IXth Leukocyte Culture Conference, 1974, p 53, #141, (abstract).

403. Massey, R.J., Levin, A. C., Johnson, D. and Deinhardt, F.: Lymphocytotoxicity to a cancer cell line: role of T and B lymphocytes. IXth Leukocyte Culture Conference 1974, p 53, #143, (abstract).

404. Mathes, L.E.,Essex, M., Yohn, D.S. and Olsen,R.G.: Characterization of cytolytic activity of cat sera having fluorescent antibody to feline oncornavirus cell membrane associated antigen. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

405. Maurer, B.A., Siwarski, D.F., Williams, J.A. and McCoy, J.L.: The establishment of an assay for human leukocyte inhibition factor (LIF). Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

406. Mayer, R.J., Smith, R.G., Sarngadharan, M.G. and Gallo, R.C.: Induction of DNA metabolizing enzymes in normal human blood lymphocytes following stimulation with phytohemagglutinin (PHA). 17th Annu Mtg of Am Soc Hematol, Atlanta, Ga., Dec. 1974, p 61, (abstract).

407. McAllister, R.M.: Candidate primate and human type C viruses. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

408. McCaffrey, R., Harrison, T.A. and Baltimore, D.: Terminal deoxynucleotidyl transferase in human bone marrow. Blood, Sept. 1974.

409. McCormick, J.J., Calhoun, L. and Rich, M.A.: Detection of reverse transcriptase in human milk: A problem. Nature 251(5477): 737-740, Oct. 1974.

410. McCormick, J.J., Larson, L. and Rich, M.A.: Studies on reverse transcriptase activity in human milk. IX Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, p 82, (abstract).

411. McCormick, J.J., McGrath, C., DeSostoa, A. and Rich, M.A.: Characterization by DNA hybridization of an oncornavirus-like particle from a human cell line derived from a malignant breast adenocarcinoma. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, p 305, (abstract).

412. McCoy, J.L., Jerome, L.F., Dean, J.H., Alford, T.C. and Herberman, R.B.: Inhibition of leukocyte migration by tumor associated antigens in soluble extracts of human breast cancer. J Natl Cancer Inst 53: 11-17, 1974.

413. McGrath, C.M., Furmanski, P., Grant, P.M., Soule, H.D., Glancy, T. and Rich, M.A.: Characterization of a candidate human oncornavirus from breast carcinoma cells in culture. IX Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, pp 84, (abstract).

414. McGrath, C.M., Furmanski, P., Soule, H., Grant, P., Longley, C. and Rich, M.A.: Immunological relationships between MuMTV, 734B and a virus-like particle isolated from human milk. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

415. McGrath, C.M., Grant, P.M., Soule, H.D., Glancy, T. and Rich, M.A.: Replication of an oncornavirus-like particle in a human breast carcinoma cell line, MCF-7. Nature 252(5480): 247-250, Nov. 15, 1974.

416. McGrath, C.M., Grant, P., Soule, H. and Rich, M.A.: Characterization of an oncornavirus-like particle replicating in a human breast carcinoma cell line. Proc XIth Int Cancer Cong, Florence, Italy, Oct. 1974, pp 305-306, (abstract).

417. Mele, J., Glaser, R. and Nonoyama, M.: Epstein-Barr virus DNA synthesis: resistance to hydroxyurea. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

418. Mele, J., Glaser, R., Nonoyama, M., Zimmerman, J.E. and Rapp, F.: Observation on the resistance of Epstein-Barr virus DNA synthesis to hydroxyurea. Virology 62: 102-111, Nov. 1974.

419. Melendez, L.V., Hunt, R.D., Garcia, F.G., Daniel, M.D. and Fraser, C.E.O.: Prevention of lymphoma in cotton-top marmosets by herpesvirus saimiri antiserum. J Med Primatol 3: 213-220, 1974.

420. Melendez, L.V., Hunt, R.D., Garcia, F.G., Jackman, D., Cadwallader, J. and Marshall, B.M.: Herpesvirus saimiri attenuated by serial passage in dog fetal lung cell line as a "vaccine" against malignant lymphoma in owl monkeys (Aotus trivirgatus). Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 680-681, (abstract).

421. Melnick, J.L., Adam, E. and Rawls, W.E.: The causative role of herpesvirus type 2 in cervical cancer. Cancer 34: 1375-1385, 1974.

422. Melnick, J.L., Boyd, A.L. and Butel, J.S.: The DNA-transfer technique: relevance to virus-cell relationships. In: Viruses, Evolution and Cancer, Academic Press, New York, 1974.

423. Menezes, J., Leibold, W. and Klein, G.: Biological differences between different Epstein Barr Virus (EBV) strains with regard to lymphocyte transforming ability. Exp Cell Res, 1975.

424. Michalides, R., Kimball, P. and Schlom, J.: Detection of MMTV sequences in murine DNA: A method to determine the interrelationship between the various MMTV strains. IX Meeting on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, p 55 (abstract).

425. Miller, M.F., II: Particle counting of viruses. In: Principles and Techniques of Electron Microscopy. Biological Applications, Vol. 4 (M.A. Hayat, ed.) Van Nostrand Reinhold Company, New York/Cincinnati/Toronto/London/ Melbourne, Aug. 1974, pp 89-127.

426. Miller, M.F., Dmochowski, L., Maruyama, K., Scanlon, M. and Chesner, J.: Acrolein prefixation for immunoferritin and immunoperoxidase studies on RNA tumor virus antigens. Proc 32nd Annual Mtg Electron Microscopy Society of America, Aug. 1974, pp 246-247, (abstract).

427. Miller, N.R., Saxinger, W.C., Reitz, M.S., Gallagher, R.E., Wu, A.M., Gallo, R.C. and Gillespie, D.: Systematics of RNA tumor viruses and virus-like particles of human origin. Proc Natl Acad Sci USA 71: 3177-3181, Aug. 1974.

428. Minowada, J., Nonoyama, M., Moore, G.E., Rauch, A.M. and Pagano, J.S.: The presence of the Epstein-Barr viral genome in human lymphoblastoid B-cell lines and its absence in a myeloma cell line. Cancer Res 34: 1898-1903, Aug. 1974.

429. Miyagi, M. and Goodheart, C.R.: Transcription of DNA of herpesvirus saimiri and of herpes simplex virus type 2. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

430. Molnar, Z., Biberfeld, P. and Klein, G.: Immuno-fluorescence and immuno-electron microscopy on EBV-associated membrane antigen of a nasopharyngeal carcinoma derived lymphoblastoid cell line (LY-28). Eur J Cancer ID: 633-637, 1974.

431. Moore, D.H.: Comments on human breast cancer virus. IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

432. Moore, D.H.: Evidence in favor of the existence of human breast cancer virus. Cancer Res 34: 2322-2329, Sept. 1974.

433. Moore, D.H.: The mammary tumor viruses. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

434. Moore, D.H.: Viruses and breast cancer. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

435. Moorhead, P.S., Nicholls, W.W., Perkins, F.T. and Hayflick, L.: Standards of karyology for human diploid cells. J Biol Stand 2: 95-101, 1974.

436. Mühlbock, O. and Dux, A.: Histocompatibility genes and development of mammary tumours and lung tumours in the O20 and OIR mouse strains. In: Proc IX Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

437. Munoz, N. and De Thé, G.: Herpes génital et cancer utérin. Actual Gynécol, 42-48, 1974.

438. Murasko, D.M. and Lausch, R.N.: Capacity of virus hyperimmune sera to block spleen cell cytotoxicity for cytomegalovirus transformed cells. Fed Proc 34(3) April 1975 (abstract).

439. Murasko, D. and Lausch, R.N.: Cellular immune response to virus specific antigen in hamsters bearing isografts of cytomegalovirus-transformed cells. Int J Cancer 14: 451-460, 1974.

440. Murray, B.K., Benyesh-Melnick, M. and Biswal, N.: Early and late viral-specific polyribosomal RNA in herpes virus-1 and -2-infected rabbit kidney cells. Biochim Biophys Acta 361: 209-220, 1974.

441. Nagle, S.C., Jr., Brown, B.L.: Epstein-Barr virus from P3HP-1 cells grown in chemically defined medium. Appl Microbiol 28: 518-520, 1974.

442. Nakajima, K., Ono, K., and Ito, Y.: Interconversion of molecular size of the DNA polymerase from Rauscher leukemia virus. Intervirology, 332-341, 1974.

443. Nakajima, K., Ono, K., Nishikawa, S., Hieda, W., Yoshida, T., Ishimoto, A. and Ito, Y.: Interconversion of molecular size of DNA polymerase from Rauscher leukemia virus. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, p 603-605.

444. Nebert, D.W., Benedict, W.F. and Kouri, R.E.: Aromatic hydrocarbon produced tumorigenesis and the genetic differences in aryl hydrocarbon hydroxylase induction. In: Chemical Carcinogenesis, Part A. (P. Ts'o and J. DiPaolo, eds.) Marcel Dekker, New York, 1974, pp 271-288.

445. Neely, A., Sabad, A., and Kersey, J.: A mitogenic ionophore A23187 enhances calcium uptake in human lymphocytes. Fed Proc 34(3) April 1975, (abstract).

446. Nelson-Rees, W.A., Zhdanov, V.M., Hawthorne, P.K. and Flandermeyer, R.R.: HeLa-like marker chromosomes and type A variant G_6PD dehydrogenase isoenzyme in human cell cultures producing Mason-Pfizer monkey virus-like particles. J Natl Cancer Inst 53(3): 751-757, Sept. 1974.

447. Neubauer, R.H., Wallen, W.C. and Rabin, H.: Stimulation of Herpesvirus saimiri expression in the absence of evidence for type C virus activation in a marmoset lymphoid cell line. J Virol 14: 745-750, 1974.

448. Nigida, S.M., Falk, L.A., Wolfe, L.G., Deinhardt, F.W., Long, W.K. and Alford, C.A.: Cytomegalovirus: experimentally-induced infection in marmoset monkeys with a human strain (Colburn). Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

449. Nomura, S., Dunn, K.J., Mattern, C.F.T., Hartley, J.W., and Fischinger, P.J.: Revertants of mouse cells transformed by murine sarcoma virus: Flat variants without a rescuable sarcoma virus from a clone of BALB/3T3 transformed by Kirsten MSV. J Gen Virol 25: 207-218, Nov. 1974.

450. Nomura, S. and Fischinger, P.J.: Control of reversion in MSV transformed cells after single and multiple transforming cycles. In: Mechanisms of Virus Disease, Vol. I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 273-283.

451. Nonoyama, M., Kawai, Y., Huang, C.H., Pagano, J.S., Hirshaut, Y., and Levine, P.H.: Epstein-Barr virus DNA in Hodgkin's disease, American Burkitt's lymphoma, and other human tumors. Cancer Res 34: 1228-1231, May 1974.

452. Nonoyama, M. and Tanaka, A.: Plasmid DNA as a possible state of Epstein-Barr virus genomes in nonproductive cells. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

453. Nonoyama, M., Tanaka, A., Hampar, B., and Derge, J.G.: Latent genomes of Epstein-Barr virus. In: Mechanisms of Virus Disease, Vol. I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 505-514.

454. Noronha, F., Dougherty, E., Poco, A., Greis, C., Post, J., and Rickard, C.: Cytological and serological studies of a feline endogenous C-type virus. Archiv fur die gesamte Virusforschung 45, 235-248, Springer-Verlag, Oct. 1974.

455. Ochs, H.D., Storb, R., Thomas, E.D., Kolb, H.J., Graham, T.C., Mickelson, E., Parr, M. and Rudolph, R.H.: Immunologic reactivity in canine marrow graft recipients. J Immunol 113: 1039-1057, Sept. 1974.

456. O'Connor, T.E., Schiop-Stansly, P., Sethi, V.S., Hadidi, A. and Okano, P.: Antibiotics effective against oncornavirus infection and transformation of human or mouse cell cultures. 27th Annu Symp on Fundamental Cancer Research, Houston, Texas, 1974, pp 38-39, (abstract).

457. O'Connor, T.E., Schiop-Stansly, P., Sethi, V.S., Hadidi, A., and Okano, P.: Antibiotics effective in blockade of infection and transformation of human and mouse cell cultures by oncornaviruses. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, p 359, (abstract).

458. O'Connor, T.E., Schiop-Stansly, P., Sethi, V.S., Hadidi, A., and Okano, P.: Antiviral antibiotics: Inhibition of focus-formation in human or mouse cell cultures by sarcoma-inducing oncornaviruses with rifamycins. Intervirology 3: 63-83, 1974.

459. Okabe, H., Gilden, R.V., and Hatanaka, M.: Specificity of the DNA product of RNA dependent DNA polymerase in type C viruses: III. Analysis of viruses derived from Syrian hamsters. Proc Natl Acad Sci USA 71: 3278-3282, Aug. 1974.

460. Olding, L.B., Benirschke, K. and Oldstone, M.B.A.: Inhibition of mitosis of lymphocytes from human adults by lymphocytes from human newborns. Clin Immunol & Immunopath 3: 79-89, 1974.

461. Oldstone, M.B.A.: Tissue injury and diseases associated with chronic viral infections: Role of the immune response. In: Mechanisms of Virus Disease, Vol. I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 19-23.

462. Olofsson, B., Hamelin, R., Tchen, P. and Tavitian, A.: Presence d'une activité RNase H non associée à l'activité transcriptase inverse dans les virions du sarcoma murin de Moloney. Comptes Rendus Acad Sci 278: 2851-2854, 1974.

463. Olpin, J., Oroszlan, S., and Gilden, R.V.: Biophysical-immunological assay for ribonucleic acid type C viruses. Appl Microbiol 28: 100-105, July 1974.

464. Olsen, R.G., Kahn, D., Hoover, E., Saxe, N., and Yohn, D.S.: Differences in clinical and convalescent phase antibodies in cats infected with feline picornavirus. Infect Immun 10: 375-380, Nov. 1974.

465. Olsen, R.G., Krakowka, S., Mathes, L.E., and Yohn, D.S.: Composite complement: A mixture of cat C1 and guinea pig complement as a reagent compatible with cat antibodies. Am J Vet Res 34: 1529-1532, Nov. 1974.

466. Olsen, R.G., Mathes, L.E., and Yohn, D.S.: Complement-fixation-inhibition as a test for antibodies in cats and humans to C-type RNA tumor virus antigen. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 373-383.

467. Orme, T.W., McCaw, B.A., Boone, C.W., Boyd, A.L.: Cellular localization in HSV-2 associated antigens in transformed mouse cells. 7th Annual Miami Winter Symp, Vols 9 & 10, Miami, Fla., Jan. 1975 (abstract).

468. Oroszlan, S., Bova, D., and Gilden, R.V.: Isolation of mammalian type C RNA virus cross-reactive antigens and antibody by immunoaffinity chromatography. Immunochem 12: 61-66, Jan. 1975.

469. Oroszlan, S., Summers, M.R., Foreman, C., and Gilden, R.V.: Murine type C virus group-specific antigens: Interstrain immunochemical, biophysical, and amino acid sequence differences. J Virol 14: 1559-1574, Dec. 1974.

470. Otten, A., Quarles, J.M., and Tennant, R.W.: Activation of murine leukemia virus by gamma irradiation. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

471. Pagano, J.S.: The Epstein-Barr viral genome and its interactions with human lymphoblastoid cells and chromosomes. In: Viruses, Evolution and Cancer. (E. Kurstak and K. Maramorosch, eds.) Academic Press Inc., 1974, pp 79-116.

472. Pagano, J.S.: The Epstein-Barr virus and malignancy: Molecular evidence. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

473. Pagano, J.S., and Huang, E.S.: The application of RNA-DNA cytohybridization to viral diagnostics. In: Viral Immunodiagnosis. (E. Kurstak and R. Morisset, eds.) Academic Press, 1974, pp 279-299.

474. Panem, S. and Schauf, V.: Cell-cycle dependent appearance of murine leukemia-sarcoma virus antigens. J Virol 13(6): 1169-1176, June 1974.

475. Panet, A., Verma, I.M., and Baltimore, D.: Role of the subunits of the avian RNA tumor virus reverse transcriptase. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

476. Pang, R.H.L., Phillips, L.A., and Park, J.J.: Nucleotide sequences in the RNA of mammalian oncornaviruses. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

477. Paran, M., Wu, A.M., Richardson, L.S. and Gallo, R.C.: Glucogenic corticosteroids and murine type C virus production. In: Symp on Modern Trends in Human Leukemia: Biological, Biochemical and Virological Aspects, 1973, (R. Neth, ed.) Wilsede, West Germany, pp 197-205, 1974.

478. Pardoe, G.I.: Heterophile receptors of erythrocytes and leukocytes. Ann NY Acad Sci 234-239, 1974.

479. Parks, W.P. and Scolnick, E.M.: Immunological cross-reactions between two low molecular weight polypeptides from a murine type C virus. J Virol 14: 430-433, 1974.

480. Parks, W.P., Scolnick, E.M. and Ransom, J.C.: Glucocorticoid induction of murine mammary tumor virus in vitro. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

481. Pazmino, N.H., Yuhas, J.M. and Tennant, R.W.: Inhibition of murine RNA tumor virus replication and oncogenesis by chloroquine. Int J Cancer 14: 379-385, 1974.

482. Perk, K., Michalides, R., Spiegelman, S., Schlom, J.: Biochemical and morphological evidence for presence of an RNA tumor virus in pulmonary carcinoma of sheep (Jaagsiekte). J Natl Cancer Inst 53: 131-135, 1974.

483. Pessac, B. and Calothy, G.: Transformation of chick embryoneuroretinal cells by Rous sarcoma virus in vitro: Induction of cell proliferation. Science 185: 709-710, Aug. 1974.

484. Peters, R.L., Rabstein, L.S., VanVleck, R., Kelloff, G.J., and Huebner, R.J.: Naturally occurring sarcoma virus of the BALB/cCr mouse. J Natl Cancer Inst 53(6): 1725-1729, Dec. 1974.

485. Peters, R.L., Spahn, G.J., Rabstein, L.S., Huebner, R.J., and Kelloff, G.J.: Sensitive in vivo assay for detection of murine leukemia viruses. Appl Microbiol 28(4): 614-617, Oct. 1974.

486. Phillips, L.A., Park, J.J., and Hollis, V.W. Jr.: Polyriboadenylate sequences at the 3'-termini of ribonucleic acid from mammalian leukemia and sarcoma viruses. Proc Natl Acad Sci USA 71:4366-4370, Nov. 1974.

487. Phillips, L.A., Park, J.J., and Pang, R.H.L.: Selective isolation of virus specific RNA from mammalian leukemogenic and sarcomogenic cells. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

488. Phillips, S.M., Gleichmann, H., Hirsch, M.S., Black, P.H., Merrill, J., Schwartz, R.S. and Carpenter, C.B.: Cellular immunity in the mouse. IV: Altered thymic dependent lymphocyte reactivity in the chronic graft vs host reaction. Cell Immunol 15: 152-168, 1975.

489. Phillips, S.M., Hirsch, M.S., Andre-Schwartz, J., Solnik, C., Black, P.H., Schwartz, R.S., Merrill, J. and Carpenter, C.B.: Cellular immunity in the mouse V: Further studies on leukemia virus activation in allogeneic reactions of mice: stimulatory parameters. Cell Immunol 15: 169-179, 1975.

490. Pike, M.C. and Smith, P.G.: Clustering of cases of Hodgkin's disease and leukemia. Cancer 34: 1390-1394, Oct. (suppl) 1974.

491. Plata, E.J., Aoki, T., and Collins, F.C.: Specificity of antibodies naturally occurring in mammary tumor (MT)-bearing and mammary tumor virus (MTV)-infected mice. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

492. Plowman, J.L., et al: Effects of C-AMP and dexamethasone on mouse mammary tumor virus-producing cells. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

493. Plummer, G., Goodheart, C.R. and Miyagi, M.: Growth of murine cytomegalovirus in a heterologous cell system and its enhancement by 5-iodo-2'-deoxyuridine. Infect Immun 10: 251-256, 1974.

494. Plummer, G., Goodheart, C.R., Miyagi, M., Skinner, G.R.B., Thouless, M.E., and Wildy, P.: Herpes simplex viruses: Discrimination of types and correlation between different characteristics. Virology 60: 206-216, 1974.

495. Prather, S.O., Sofranko, J. and Lausch, R.N.: Tumor immunity in SV40 sensitized hosts. Evidence for antibody-dependent cellular cytotoxicity. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

496. Price, P.J., Suk, W.A., Spahn, G.J., Chirigos, M.A., Lane, J.A., and Huebner, R.J.: Streptonigrin inhibition of 3-methylcholanthrene transformation in vitro. Proc Soc Exp Biol Med 145: 1197-1200, 1974.

497. Priori, E.S., and Dmochowski, J.: Immunofluorescence antibodies in human sera to antigens in cells of a type C virus producing human culture of tumor origin. In: Immunology of Cancer. Progr. Exp. Tumor Res Vol 19 (V. Richards, ed)Karger, Basel, 1974, p 182-193.

498. Priori, E.S., East, J.L. and Dmochowski, L.: Transformation of cells in human bone tumor cultures. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

499. Priori, E.S., Peterson, C., Ilyin, K. V. and Dmochowski, L: Immunological studies on the relationship between human breast carcinoma and oncornavirus isolate from HEp-2 cells. In: Proc IX Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, p 28-30 (abstract).

500. Prival, J.T., Paran, M., Gallo, R.C. and Wu, A.M.: Colony stimulating factors produced from human peripheral blood cells in culture. J Natl Cancer Inst 53: 1583-1588, 1974.

501. Proffitt, M.R., Hirsch, M.S. and Black, P.H.: Autoreactivity and leukemogenesis. In: Mechanisms of Virus Disease, Vol. I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 3-12.

502. Puga, A., Jenson, A.B., Boaz, J., Jenson, F., Kohne, D.E. and Lerner, R.A.: Molecular analysis of a murine leukemia virus produced by continuously growing thymocytes. In: Progress in Immunology II, Vol. 5(L. Brent and J. Holborow, eds.) North-Holland/ American Elsevier, Amsterdam/New York, 1974, pp 15-25.

503. Puga, A., Jenson, A., Jensen, F., Kohne, D., and Lerner, R.: Physical and biological properties of MuLV (Scripps): Variations dependent on cellular origin. Fed Proc 34(3) April 1975, (abstract).

504. Quarles, J.M. and Tennant, R.W.: Effects of nitrosocarbaryl on cell transformation and RNA tumor virus expression of BALB/3T3 mouse cells. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

505. Rabin, H: Oncornaviruses of nonhuman primate origin. In: CRC Handbook on Laboratory Animal Science (E.C. Melby, Jr. and N.H. Altman, eds.) CRC Press, Inc., 1974, pp 39-45.

506. Rabin, H., Neubauer, R.H., Pearson, G.R., Cicmanec, J.L., Wallen, W.C., Loeb, W.F. and Valerio, M.G.: Brief communication: Spontaneous lymphoma associated with Herpesvirus saimiri in owl monkeys. J Natl Cancer Inst 54: 1975.

507. Rabin, H., Pearson, G.R., Wallen, W.C., Neubauer, R.H., Cicmanec, J.L. and Orr, T.W.: Infection of capuchin monkeys (Cebus albifrons) with Herpesvirus saimiri. J Natl Cancer Inst 54: 1975.

508. Rand, K.H., Long, C.W., Wei, T.T., and Gilden, R.V.: Plaque assay for the Mason-Pfizer monkey virus. J Natl Cancer Inst 53: 449-452, Aug. 1974.

509. Randolph, V.L. and Heath, C.W., Jr.: Influenza during pregnancy in relation to subsequent childhood leukemia and lymphoma. Am J Epidemiol 100: 399-409, 1974.

510. Rapaport, E. and Zamecnik, P.C.: A new chemical procedure for ^{32}P-labeling of ribonucleic acids at their 5'-ends after isolation. Proc Natl Acad Sci USA 72: 314-317, 1975.

511. Rapp, F.: The association of herpesviruses with cancer. The Am Col Physicians, Univ of Mich Med Cen, Ann Arbor, Mich., Current Concepts in Oncology, Feb. 1975, p 2, (abstract).

512. Rapp, F.: Demonstration of the oncogenic potential of herpes simplex viruses and cytomegalovirus. Proc Cold Spring Harbor Symposium on Quantitative Biology, Cold Spring Harbor, N.Y., May 30-June 6, 1974, (abstract).

513. Rapp, F.: Herpesviruses and cancer. In: Advances in Cancer Research, Vol 19, (G. Klein and S. Weinhouse, eds.), Academic Press, Inc., New York, N.Y., 1974, pp 265-302.

514. Rapp, F.: Transformation of mammalian cells to malignancy by human herpes viruses. In: Advances in the Biosciences 12 (G. Raspe, ed.), Pergamon Press-Vieweg, 1974, pp 605-615.

515. Rapp, F.: Virus-mediated transformation of mammalian cells. In: Developmental Aspects of Carcinogenesis and Immunity (T.J. King, ed.) Academic Press, Inc., N.Y., 1974, pp 125-136.

516. Rapp, F. and Buss, E.R.: Are viruses important in carcinogenesis? Am J Pathol 77: 85-102, 1974.

517. Rapp, F. and Duff, R.: Oncogenic conversion of normal cells by inactivated herpes simplex viruses. Cancer 34(4): 1353-1362, Oct. 1974.

518. Rapp, F., Haspel, M.V., Duff, R. and Knight, P.: Measles virus latency and neurovirulence. In: Mechanisms of Virus Disease, Vol I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds.) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 199-212.

519. Rapp, F. and Koment, R.W.: Herpesvirus etiology of abnormal growth. In: Viruses, Evolution and Cancer (E. Kurstak and K. Maramorosch, eds.) Academic Press, Inc., New York, N.Y., 1974, pp 183-205.

520. Rapp, F. and Li, J.H.: Demonstration of the oncogenic potential of herpes simplex viruses and human cytomegalovirus. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

521. Rapp, F. and Mellors, A.J.: The oncogenic properties of herpesviruses. In: Pathobiol Annu 1974 (H.L. Ioachim, ed.) Appleton-Century-Crofts, New York, N.Y., 1974, pp 101-128.

522. Rapp, F. and Reed, C.L.: Experimental evidence that herpes simplex virus type 2 is an oncogenic virus. Cervical Herpes Meeting, Bethesda, Md., April 7-9, 1975, (abstract).

523. Rapp, F. and St. Jeor, S.C.: Transformation by herpes simplex and cytomegalovirus. In: Viral Transformation and Endogenous Viruses (A.S. Kaplan, ed.) Academic Press, Inc., New York, N.Y., 1974, pp 35-53.

524. Rapp, F. and Sanford, E.: Herpesviruses and human prostatic neoplasia. Natl Prostatic Cancer Project Workshop, Natl Cancer Inst Natl Organ Site Programs, Roswell Park Memorial Institutes, Buffalo, N.Y., 1974, p 6, (abstract).

525. Rawls, W.E., Adam, E., Smith, J.W., and Melnick, J.L.: Antibodies to herpesvirus types 1 and 2 and cervical cancer. In: Molecular Studies in Viral Neoplasia, 25th Annual Symp on Fundamental Cancer 1972, Univ of Texas, M.D. Anderson Hosp and Tumor Inst., Houston, (Williams and Wilkins Co., Baltimore, Md.) 1974, p 608.

526. Reisinger, R.C.: A final mechanism of cardiac and respiratory failure. In: SIDS 1974, (R.R. Robinson, ed.) pp 77-82.

527. Reitz, M.S., Smith, R.G. and Gallo, R.C.: Endogenous RNase-sensitive DNA synthesis is directed by DNA in particulate fractions from normal human lymphocytes. Panel presentation on Enzymes of Nucleic Acid Metabolism, Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, p 314, (abstract).

528. Reitz, M.S., Smith, R.G., Roseberry, E.A. and Gallo, R.C.: DNA-directed and RNA-primed DNA synthesis in microsomal and mitochondrial fractions of normal human lymphocytes. Biochem Biophys Res Commun 57: 934-948, 1974.

529. Ressang, A.A., Mastenbroek, N., Quak, J., Van Griensven, L.J.L.D., Calafat, J., Hilgers, J., Hageman, Ph. C., Souissi, T., and Swen, S.: Studies on bovine leukemia. I. Establishment of type C virus producing cells. Vet Med B 21: 602-617, 1974.

530. Rhim, J.S.: Combined chemical and RNA viral carcinogenesis. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

531. Rhim, J.S., Cho, H.Y., and Huebner, R.J.: Nonproducer cells of murine sarcoma virus transformed human cells. Int J Cancer 15: 23-29, 1975.

532. Rhim, J.S., Ro, H.S., Kim, E.B., Gilden, R.V., and Huebner, R.J.: Transformation of horse skin cells by type C sarcoma viruses. Int J Cancer 15: 555, Feb. 1975.

533. Rhim, J.S., Wuu, K.D., Ro, H.S., Vernon, M.L. and Huebner, R.J.: Induction of guinea pig leukemia-like virus from cultured guinea pig cells. Proc Soc Exp Biol Med 147: 323-330, Oct. 1974.

534. Rhim, J.S., Yajima, Y., Ricklis, S., Huebner, R.J. and Gilden, R.V.: Consecutive activation of a type C RNA and polyoma virus from tumors induced by polyoma virus-transformed rat cells. Proc Soc Exp Biol Med 147: 730-735, Dec. 1974.

535. Rho, H.M. and Green, M.: The homopolyadenylate and adjacent nucleotides at the 3'-terminus of 30-40S RNA subunits in the genome of murine sarcoma-leukemia virus. Proc Natl Acad Sci USA 71: 2386-2390, 1974.

536. Rich, M.A., Das, M.R., Furmanski, P., McCormick, J.J., McGrath, C., Russo, J., Soule, H. and Brennan, M.: Studies on the role of viruses in human breast cancer. Annual Mtg Breast Cancer Task Force, Feb. 1975, (abstract).

537. Riggs, J.L., McAllister, R.M. and Lennette, E.H.: Immunofluorescent studies of RD-114 virus replication in cell culture. J Gen Virol 25: 21-29, 1974.

538. Robert-Robin, J., Dmanoil-Pavicovitch, R., Bazilier, M. and Boiron, M.: Isolation and characterization of "8S" RNA in murine sarcoma virus infected cells. Biochem Biophys Res Commun 60(3); 965-975, 1974.

539. Rose, W. and Girardi, A.J.: Importance of age of host in detecting tumor-associated transplantation antigens. J Immunol 113: 1058-1060, 1974.

540. Roy-Burman, P., Pal, B.K., Gardner, M.B. and McAllister, R.M.: Structural polypeptides of primate derived type C RNA tumor viruses. Biochem Biophys Res Commun 56: 543-551, 1974.

541. Roy-Burman, P., Pal, B.K., Kaplan, M., Wright, M. and Gardner, M.B.: Biochemical properties of wild mouse oncornaviruses with lymphomagenic and neurotropic activities. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

542. Royston, I., Smith, R.W., Buell, D.N., Pearson, G.R., Huang, E.S., and Pagano, J.S.: Autologous human B and T lymphoblastoid cell lines. Nature 251: 745-746, Oct. 1974.

543. Rubio, V. and Long, C.: Synthesis of a DNA binding protein in normal and transformed cells. Int J Cancer 14: 348-358, Sept. 1974.

544. Russo, I., Saby, J. and Russo, J.: Pathogenesis of rat mammary carcinomas induced by DMBA. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

545. Russo, J. and Furmanski, P.: Differentiation of human breast epithelial cells in culture. J Cell Biol 63(2): 292a, Nov. 1974, (abstract).

546. Russo, J. and McGrath, C.: Scirrhous carcinoma in the mouse: A model for human mammary carcinoma. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 468-469, (abstract).

547. Russo, J. and Soule, H.: Reproduction of the tumor histological pattern by a human carcinoma cell line (MCF-7) in sponge culture. IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, p 43, (abstract).

548. Sabin, A.B. and Tarro, G.: Herpes simplex and herpes genitalis viruses in etiology of some human cancers. Excerp Med Virol 4(9):620, 1974.

549. St. Jeor, S.C., Hutt, R. and Rapp, F.: Relationship between human cytomegalovirus and cell DNA synthesis. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

550. Sarin, P.S., Anderson, P.N., Gallo, R.C.: Terminal transferase (TT) in blast phase chronic myelogenous leukemia (CML). Amer Soc Hematol, Dec. 1974, (abstract).

551. Sarin, P.S., Reitz, M.S. and Gallo, R.C.: Transcription of heteropolymeric regions of avian myeloblastosis virus high molecular weight RNA with E. coli DNA polymerase I. Biochem Biophys Res Commun 59: 202-214, 1974.

552. Sarma, P.S. and Gazdar, A.F.: Recent progress in studies of mouse type C viruses. Current Topics in Microbiol and Immunol 68: 1-28, Dec. 1974.

553. Sarma, P.S., Jain, D., Hill, P.R.: In vitro host range of feline leukemia virus. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 437-440.

554. Sarma, P.S., Sharar, A., Tseng, J., Price, P.J., and Gardner, M.B.: Studies on the prevalence of endogenous type C virus RD114 in cats. Proc Soc Exp Biol Med 145: 757-762, 1974.

555. Sarngadharan, M.G., Leis, J.P. and Gallo, R.C.: Isolation and characterization of a ribonuclease from human leukemic blood cells specific for ribonucleic acid of ribonucleic acid - deoxyribonucleic acid hybrid molecules. J Biol Chem 250: 365-373, 1975.

556. Sarngadharan, M.G., Wu, A.M. and Gallo, R.C.: Separation of RNase H activity from reverse transcriptase in mammalian type C viruses and human leukemic cells. Workshop on "Viruses and Human Cancer", Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 299. (abstract).

557. Schacter, B., Hopkins, K., Bias, W.B.: Normal human sera detecting human leukemia associated antigens. 7th Annual Miami Winter Symp, Vol 7, Miami, Fla., Jan. 1975, p 102, (abstract).

558. Schafer, M.P., Chirigos, M.A., Papas, T.S.: The inhibition of Rauscher leukemia virus and avian myeloblastosis virus DNA polymerases by tilerone hydrochloride and its analogs. Cancer Chemother Rep 58: Nov.-Dec. 1974.

559. Schafer, W., Hunsmann, G., Moennig, V., de Noronha, F., Bolognesi, D.P., Green, R.W. and Hüper, G.: Polypeptides of mammalian oncornaviruses. II. Characterization of a murine leukemia virus polypeptide (p15) bearing interspecies reactivity. Virology 63: 48-59, 1975.

560. Schaffer, P.A., Brunschwig, J.P., McCombs, R.M. and Benyesh-Melnick, M.: Electron microscopic studies of temperature-sensitive mutants of herpes simplex virus type 1. Virology 62: 444-457, 1974.

561. Schaller, J.P. and Yohn, D.S.: Transformation potentials of the non-infectious (defective) component in pools of adenovirus type 12 and SA-7. J Virol 14: 392-401, 1974.

562. Schauf, V., Kruse, R. and Deinhardt, F.: BCG immunization in marmoset monkeys. J Med Primatol 3(5): 315-324, 1974.

563. Schidlovsky, G. and Ahmed, M.: Type C particles in mice, monkeys and humans. Proc Symp on Characterization of Oncorna and Related Viruses, Washington, D.C., Sept. 1974.

564. Schidlovsky, G., Alessi, A., Lamzardi, G., Green, W., Ahmed, M. and Korol, W.: Viral studies on human breast and placental tissues. IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

565. Schlom, J., Colcher, D., Schochetman, G., Michalides, R., Boehm-Truitt, M., and Kimball, P.: Biochemical studies of Mason-Pfizer monkey virus. IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, p 80, (abstract).

566. Schlom, J., Colcher, D., Schochetman, G., Michalides, R., Boehm-Truitt, M., and Kimball, P.: Biochemical studies of Mason-Pfizer monkey virus. Proc Symp on Characterization of Oncorna and Related Viruses, Washington, D.C., Sept. 1974, p 46, (abstract).

567. Schlom, J., Michalides, R., Colcher, D., Feldman, S., Spiegelman, S.: Evidence for an RNA tumor virus in human milk. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 419-430.

568. Schlom, J. and Spiegelman, S.: Breast cancer: molecular basis for a viral etiology. N.Y. State J Med, 1974, pp 1373-1384.

569. Schluter, B., Tennant, R.W., Yang, W.K. and Brown, A.: Fv-1 gene inhibition of mouse leukemia virus: Further characterization of the gene product(s). Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

570. Schochetman, G., Kimball, P. and Schlom, J.: RNA subunit and protein structure of Mason-Pfizer monkey virus. IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, p 78, (abstract).

571. Scolnick, E.M., Goldberg, R.J. and Parks, W.P.: A biochemical and genetic analysis of mammalian RNA containing sarcoma viruses. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

572. Scolnick, E.M., Maryak, J.M. and Parks, W.P.: Levels of rat cellular RNA homologous to either Kirsten sarcoma virus or rat type C virus in cell lines derived from Osborne-Mendel rats. J Virol 14: 1435-1444, 1974.

573. Seman, G.: Search for tumor viruses by electron microscopy. Cancer Bulletin 26(4): 74-79, July-Aug. 1974.

574. Seman, G., and Dmochowski, L.: Ultrastructural characteristics of human tumor cells in vitro. In: Human Tumor Cells in Vitro (J. Fogh, ed.) Plenum Publishing Co., New York, 1974.

575. Seman, G., Dmochowski, L., and Hunter, S.: Virus particles in placenta and uterus of RIII/Dm strain mice. Proc 32nd Annual Mtg Electron Microscopy Society of America, Aug. 1974, pp 248-9, (abstract).

576. Seman, G., Hunter, S.J., and Dmochowski, L.: Two established cell lines (SH-3 and SH-3) derived from human breast cancer. In: Proc IXth Mtg on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, p 28-30, (abstract).

577. Seman, G., Hunter, S.J., Lukeman, M.J., and Dmochowski, L.: Observations on two cell lines (SH-3 and SH-3) derived from breast cancer. Proc Ann Mtg SW Section AM Soc Cancer Res, New Orleans, La., Nov. 8-9, 1974, p 45-46, (abstract).

578. Sessions, R.B., Goepfert, H., Adam, E. and Rawls, W.E.: Herpesvirus and Epstein-Barr virus antibodies and carcinoma of the intrinsic larynx. Archives Otolaryngol 99: 261-263, 1974.

579. Seth, P., Rawls, W.E., Duff, R., Rapp, F., Adam, E. and Melnick, J.L.: Antigenic differences between isolates of herpesvirus type 2. Intervirology 3: 1-14, 1974.

580. Sethi, V.S. and Gallo, R.C.: Deoxyribonucleic acid-dependent ribonucleic acid polymerases from spleen of uninfected and Rauscher murine leukemia virus-infected NIH Swiss mice. Biochim Biophys Acta 269: 269-281, 1975.

581. Shah, K.V., Rangan, S.R.S., Reissig, M. and Roberts, J.: A new papovavirus from stumptailed macaques (Macaca speciosa) with unusual biologic features. Fed Proc 34(3) April 1975, (abstract).

582. Shanmugam, G., Bhaduri, S., Arens, M., and Green, M.: DNA-binding proteins in the cytoplasm and in a nuclear membrane complex isolated from uninfected and adenovirus 2 infected cells. Biochem 14(2): 332-337, 1975.

583. Shantz, G.D. and Lausch, R.N.: Variation in quantitative expression of Forssman antigen on virus transformed hamster cells. J Natl Cancer Inst 53: 239-246, 1974.

584. Shapiro, S.Z., Strand, M.: High molecular weight precursor proteins of Rauscher murine leukemia virus. Fed Proc 34(3) April 1975, (abstract).

585. Sheffield, J.B., Lasfargues, E.Y., and Daly, T.M.: Studies on the synthesis of MuMTV in a continuous cell line. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

586. Sherr, C.J., Benveniste, R.E. and Todaro, G.J.: Type C viral expression in primate tissues. Proc Natl Acad Sci USA 71: 3721-3725, Sept. 1974.

587. Sherr, C.J. and Todaro, G.J.: Radioimmunoassay of the major group specific protein of endogenous baboon type C viruses: Relation to the RD-114/CCC group and detection of antigen in normal baboon tissues. Virology 61: 168-181, Sept. 1974.

588. Sherr, C.J. and Todaro, G.J.: Type C viral antigens in man. I. Antigen related to endogenous primate virus in human tumors. Proc Natl Acad Sci USA 71: 4703-4707, Dec. 1974.

589. Shibley, G.P., Robinson, O.R., Johnson, R.W., Perry, A., Bandyopadhyay, A.K.: Factors affecting RNA-dependent DNA polymerases (RDDP), activity in large-scale production and purification of murine (RLV) and primate (SSV-1) virus propagated in cell culture. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

590. Shoyab, M.. Evans, R.M. and Baluda, M.A.: Presence in leukemic cells of avian myeloblastosis virus-specific DNA sequences absent in normal chicken cells. J Virol 14: 47-49, 1974.

591. Shoyab, M., Markham, P.D. and Baluda, M.A.: Reliability of the RNA-DNA filter hybridization for the detection of oncornavirus-specific DNA sequences. J Virol 14: 225-230, 1974.

592. Siegler, R. and Benjamin, T.: Oncogenicity of wild type and mutant strains of polyoma virus. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

593. Silberstein, H. and August, J.T.: A virion protein kinase: Purification and properties. Fed Proc 34(3) April 1975, (abstract).

594. Silvestre, D., Ernberg, I., Neauport-Sautes, C., Kourilsky, F.M. and Klein, G.: Differentiation between early and late membrane antigen on human lymphoblastoid cell lines infected with Epstein-Barr virus. II. Immunoelectron microscopy. J Natl Cancer Inst 53: 67-74, 1974.

595. Simons, M.J., Wee, G.B., Day, N.E., Chan, S.H., Shanmugaratnam, K., and De Thé, G.: Immunogenetic aspects of nasopharyngeal carcinoma (NPC): IV. Probable identification of an HL-A second antigen associated with a high risk for NPC. Lancet 1; 142-143, 1975.

596. Singh, M., Singh, V.N., August, J.T. and Horecker, B.L.: Mechanisms of alterations in glucose metabolism in Rous sarcoma virus-transformed chick embryo cells: Transformation-specific changes in the activities of key enzymes of the glycolytic and hexose monophosphate shunt pathways. Arch Biochem Biophys 165, 240-246, 1974.

597. Singh, V.N., Singh, M., August, J.T. and Horecker, B.L.: Alterations in glucose metabolism in chick-embryo cells transformed by Rous sarcoma virus: Intracellular levels of glycolytic intermediates. Proc Natl Acad Sci USA 71: 4129-4132, 1974.

598. Sinkovics, J.G., Kay, H.D. and Clark, R.L.: Are antiviral agents useful in the treatment of malignant tumors? Cancer Bulletin, 26: 69-73, 1974.

599. Sinkovics, J.G., Williams, D.E., Campos, L.T., Kay, H.D. and Howe, C.D.: Metastatic sarcomas: Chemotherapy and sarcoma-specific immune reactions. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 289-290, (abstract).

600. Sinkovics, J.G., Williams, D.E., Campos, L.T., Kay, H.D. and Romero, J.J.: Intensification of immune reactions of patients to cultured sarcoma cells: Attempts at monitored immunotherapy. Oncology 1: 351-365, 1974.

601. Sinkovics, J.G., Williams, D.E., Kay, H.D., Campos, L.T. and Howe, C.D.: Clinical settings favorable for immunotherapy of sarcomas. Interaction of Radiation and Host Immune Defense Mechanisms in Malignancy Conference, March 1974, Greenbrier, W. Va., Brookhaven Natl Lab Rep 50418, pp 331-339, 1974.

602. Smith, P.G., Pike, M.C., Kinlen, L.J. and Jones A.: Recent studies of person-person transmission of Hogkin's disease. Proc Roy Soc Med 67: 683-684, July 1974.

603. Soeiro, R., Sveda, M.M., Krontiris, T.G., and Fields, B.N.: Host restriction of Friend leukemia virus. In: Mechanisms of Virus Disease, Vol. I, ICN/UCLA Symposia on Molecular and Cellular Biology (W.S. Robinson and C.F. Fox, eds) W.A. Benjamin, Inc., Menlo Park, California, 1974, pp 69-86.

604. Sottong, P., Hill, P., Feeney, M., Klecker, J., Johnson, K., Harris, R., Bell, C., and Stafford, K.: Recovery of murine leukemia virus from large volumes of freshly harvested culture fluids by using a single density gradient. Appl Microbiol 29(1): 102-105, Jan. 1975.

605. Souissi, T., Hilgers, J., Verstraeten, A., Kwa, H., and Van Nie, R.: Mammary tumor virus antigen expression in genital tract organs of male mice from low and high mammary cancer strains. In: Proc IX Meeting on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

606. Spiegelman, S., Axel, R., Baxt, W., Kufe, D., and Schlom, J.: Human cancer and animal viral oncology. Cancer 34: 1406-1420, 1974.

607. Spiegelman, S., Baxt, W., Kufe, D., Peters, W.P. and Schlom, J.: Sequences related to the RNA tumor viruses in the RNA and DNA of human leukemias and lymphomas. In: Comparative Leukemia Research 1973, VIth Int Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975, pp 1-25.

608. Spiegelman, S., Schlom, J., Axel, R., Hehlmann, R., and Kufe, D.: Molecular probing for a viral etiology of human cancer. In: Molecular Studies in Viral Neoplasia. The Williams & Wilkins Co., 1974, pp 547-573.

609. Springer, E.L., Hackett, A.J., and Byers, V.S.: Scanning and transmission electron microscopy of human T cell mediated cytolysis. 7th Annual Miami Winter Symp, Vols 9 & 10, Miami, Fla., Jan. 1975, (abstract).

610. Springer, E.L., Riggs, J.R., and Hackett, A.J.: Viral identification by scanning electron microscopy of preparations stained with fluorescein-labeled antibody. J Virol 14(6): 1623-1626, Dec. 1974.

611. Stavnezer, J., Huang, R.C.C., Stavnezer, E., and Bishop, J.M.: Isolation of mRNA for an immunoglobulin kappa chain and enumeration of the genes for the constant region of kappa chain in the mouse. J Mol Biol 88(1): 43-63, Sept. 1974.

612. Steeves, R.A., Strand, M. and August, J.T.: Structural proteins of mammalian oncogenic RNA viruses: Murine leukemia virus neutralization by antisera prepared against purified envelope glycoprotein. J Virol 14: 187-189, July 1974.

613. Steiner, M.R., Somers, K., and Steiner, S.: Incorporation of (^{14}C)-2 deoxy-D-glucose into the lipids of normal cells as compared to virus-transformed cells. Biochem Biophys Res Commun 61: 795-801, 1974.

614. Steiner, S. and Melnick, J.L.: Altered fucolipid patterns in cultured human cancer cells. Nature 251: 717-718, 1974.

615. Stephens, R., Traul, K., Lowry, G., Woolf, D., and Lelek, J.: Improved recognition and quantitation of oncornaviruses using BEEM capsules. Proc 32nd Annual Mtg Electron Microscopy Society of America, Aug. 1974, pp 12-13.

616. Stephens, R., Traul, K., Woolf, D., Lowry, G., and Lelek, J.: An improved recognition and quantitation technique for oncornaviruses. J Clin Microbiol Vol. 1(2), Feb. 1975.

617. Stephenson, J.R. and Aaronson, S.A.: Demonstration of a genetic factor influencing spontaneous release of a xenotropic virus of mouse cells. Proc Natl Acad Sci USA 71: 4925-4929, Dec. 1974.

618. Stephenson, J.R., Aaronson, S.A., Arnstein, P., Huebner, R.J., and Tronick, S.R.: Demonstration of two immunologically distinct xenotropic RNA type C viruses of mouse cells. Virology 61: 244-248, Sept. 1974.

619. Stephenson, J.R., Crow, J.D., and Aaronson, S.A.: Differential activation of biologically distinguishable endogenous mouse type C RNA viruses. Interactions with host cell regulatory factors. Virology 61: 411-419, Oct. 1974.

620. Stephenson, J.R., Tronick, S.R., and Aaronson, S.A.: Isolation from BALB/c mouse cells of a structural polypeptide of a third endogenous type C virus class. Cell 3: 347-353, Dec. 1974.

621. Stephenson, J.R., Tronick, S.R., and Aaronson, S.A.: Temperature sensitive mutants of murine leukemia virus. IV. Further physiologic characterization and evidence for genetic recombination. J Virol 14: 918-923, Aug. 1974.

622. Storb, R., Epstein, R.B., Graham, T.C., Kolb, H.J., Kolb, H., and Thomas, E.D.: Rescue from canine graft-versus-host reaction by autologous or DL-A compatible marrow. Transplantation 18: 357-367, 1974.

623. Storb, R., Gluckman, E., Thomas, E.D., Buckner, C.D., Clift, R.A., Fefer, A., Glucksberg, H., Graham, T.C., Johnson, F.L., Lerner, K.G., Neiman, P.E., and Ochs, H.: Treatment of established human graft-versus-host disease by antithymocyte globulin. Blood 44: 57-75, July 1974.

624. Storb, R., Thomas, E.D., Buckner, C., Clift, R.A., Fefer, A., Glucksberg, H., and Neiman, P.E.: Transplantation of bone marrow in refractory marrow failure and neoplastic disease. Am J Clin Pathol 62: 212-217, 1974

625. Strand, M. and August, J.T.: Structural proteins of RNA tumor viruses as probes for viral gene expression. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

626. Strand, M. and August, J.T.: Type C RNA virus gene expression in human tissue. J Virol 14: 1584-1596, 1974.

627. Strand, M., Lilly, F., August, J.: Genetic control of the expression of murine leukemia virus proteins in tissues of normal, young adult mice. In: Cold Spring Harbor Symposium, Vol. XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

628. Strand, M., Lilly, F., August, J.: Host control of endogenous murine leukemia virus gene expression: Concentrations of viral proteins in high and low leukemia mouse strains. Proc Natl Acad Sci USA 71: 3682-3686, Sept. 1974.

629. Strand, M., Wilsnack, R. and August, J.T.: Structural proteins of mammalian oncogenic RNA viruses: Immunologic characterization of the p15 polypeptide of Rauscher murine virus. J Virol 14(6): 1575-1583, Dec. 1974.

630. Sulzer, M.J., Strobel, P.L., Springer, E.L., Roth, I. and Calloway: A comparative electron microscopic study of the morphology of Toxoplasma gondii by freeze etch replication and then sectioning technique. J Protozoology 15: 326-336, 1974.

631. Sveda, M.M., Fields, B.N., and Soiero, R.: Host restriction of Friend leukemia virus: Fate of input virion RNA. Cell (2): 271-272, Aug. 1974.

632. Svedmyr, E.A., Deinhardt, F., Gatti, R.A., Golub, S., Gunvén, P., Hoekstra, J., Klein, G., Leibold, W., Menezes, J., and Wigzell, H.: Sensitization of human lymphocytes with autologous lymphoblastoid cell line. In: Proc 8th Leucocyte Culture Conf., Uppsula, 1973, Academic Press, pp. 217-222, 1974.

633. Svedmyr, E.A., Deinhardt, R., Golub, S., Gunvén, P., Hoekstra, J. and Klein, G.: Studies on specific lymphocyte activity in Burkitt lymphoma and infectious mononucleosis. In: Advances in the Biosciences 12 (G. Raspe and S. Bernhard, eds.) Pergamon Press-Vieweg, 1974, pp 511-522.

634. Svedmyr, E.A., Deinhardt, F., and Klein, G.: Sensitivity of different target cells to the killing action of peripheral lymphocytes stimulated by autologous lymphoblastoid cell lines. Int J Cancer 13: 891-903, 1974.

635. Svedmyr, E., Wigzell, H., and Jondal, M.: Sensitization of human lymphocytes against autologous or allogeneic lymphoblastoid cell lines: Characteristics of the reactive cells. Scand J Immunol 3: 499-508, 1974.

636. Szakacs, J.E., Carta, M.A., Szakacs, M.R.: Ewing's sarcoma extraskeletal and of bone. Case report with ultrastructural analysis. Ann Clin Lab Sci 4(4): 306-322, July 1974.

637. Szakacs, J.E., Szakacs, M.R.: Search for C-type particles in human neoplasia. Ann Clin Lab Sci 5(1): 14-22, Jan. 1975.

638. Takasugi, M. and Kinoshita, K.: Specific antibody activation of lymphocytes for cell-mediated cytotoxicity against cultured target cells. J Natl Cancer Inst 53: 1539-1544, 1974.

639. Tamerius, J.D. and Hellström, I.: In vitro demonstration of cytotoxic antibodies to Moloney sarcoma cells. J Immunol 112, 1987-1996, 1974.

640. Tanaka, A. and Nonoyama, M.: Latent DNA of Epstein-Barr virus: Separation from high molecular weight cell DNA in a neutral glycerol gradient. Proc Natl Acad Sci USA 71(12): 4658-4661, Dec. 1974.

641. Tarro, G.: Cancer research and role of viruses. In: The Leonard. (S. Vagnoni, ed.) E.N.B.P.S., Rome 1974, pp 270-276.

642. Tarro, G. and Battista, A.: Further studies on antibodies for herpesvirus nonvirion antigens and presence of herpesvirus genetic markers in cancer patients. 8th Mtg European Tumor Virus Group, Aviemore, Scotland, April 1974, (abstract).

643. Tarro, G., Battista, A., De Nucci, C., Di Gioia, M. and Cocchiara, R.:Antibodies for herpesvirus nonvirion antigens and herpesvirus genetic markers in cancer patients. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

644. Tarro, G. and Sabin, A.B.: Nonvirion antigens produced by herpes simplex viruses 1 and 2. Excerp Med Virol 4(4): 249, 1974.

645. Tavitian, A., Hamelin, R., Tchen, P., Olofsson, B. and Boiron, M.: Extent of transcription of mouse sarcoma-leukemia virus by RNA-directed DNA polymerase. Proc Natl Acad Sci USA, 71: 755-759, 1974.

646. Taylor, J.M., Doxy, A., Kan, Y.W., Varmus, H.E., Lie-Injo, L.E., Ganesan, J., and Todd, D.: Genetic lesion in homozygous α-thalassemia (Hydrops fetalis). Nature 251(5474): 392-393, Oct. 1974.

647. Taylor, J.M., Garfin, D.E., Levinson, W.E., Bishop, J.M., and Goodman, H.M.: Tumor virus RNA-directed DNA synthesis: Nucleotide sequence at the 5'-terminus of nascent DNA. Biochemistry 13(15): 3159-3163, July 1974.

648. Tennant, R.W., Hanna, M.G., Jr. and Farrelly, J.G.: Polyadenylic acids effect autogenous immunity and susceptibility to RNA tumor virus oncogenesis in vivo. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

649. Tennant, R.W., Hanna, M.G., Jr. and Farrelly, J.G.: Polyadenylic acids effect autogenous immunity and susceptibility to RNA virus oncogenesis. Proc Natl Acad Sci USA 71: 3167-3171, Aug. 1974.

650. Tennant, R.W., Myer, F.E. and McGrath, L.M.: Effect of the Fv-1 gene on leukemia virus in mouse cell heterokaryons. Int J Cancer 14: 504-513, 1974.

651. Tennant, R.W., Schluter, B., Yang, W.K. and Brown, A.: Reciprocal inhibition of mouse leukemia virus infection by Fv-1 allele cell extracts. Proc Natl Acad Sci USA 71 (10): 4241-4245, Oct. 1974.

652. Teramoto, Y.A. and Cardiff, R.D.: The structure of the mouse mammary tumor virus. Proc Symp on Characterization of Oncorna and Related Viruses, Washington, D.C., Sept. 1974.

653. Thomas, E.D., Fefer, A., Storb, R., Clift, R.A., Johnson, F.L. and Neiman, P.E.: Marrow transplantation: Treatment for refractory aplastic anemia or acute leukemia. U.W. Med. 1: 19-24, Sept. 1974.

654. Thota, H., Romero, J.J., Sinkovics, J.G., Carrier, S.K., Crawford, J., Kay, H.D.: Leukocytes of patients with sarcomas express significantly more cytotoxicity to cultured sarcoma cells than to carcinoma cells. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

655. Thota, H., Sinkovics, J.G., Carrier, S.K., Romero, J.J., Kay, H.D.: Lymphocyte-mediated cytotoxic cross-reactivity between cultured human melanoma and sarcoma cells. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

656. Ti, M. and Dosik, H.: Acute leukemia presenting as laryngeal obstruction. Cancer 34: 427-30, Aug. 1974.

657. Ting, R.C., Smith, R.G., Gallo, R.C. and Wu, A.M.: Comparison of drug effects in RNA tumor virus-infected cells. In: Comparative Leukemia Research 1973, VIth Int. Symp on Comparative Leukemia Res., Nagoya/Ise-Shima, Sept. 1973 (Y. Ito and R.M. Dutcher, eds.) Univ of Tokyo Press, 1975.

658. Todaro, G.J., Benveniste, R.E., Callahan, R., Lieber, M. and Sherr, C.J.: Endogenous primate and feline type C viruses. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

659. Todaro, G.J., Sherr, C.J., Benveniste, R.E., Lieber, M.M. and Melnick, J.L.: Type C viruses of baboons: Isolation from normal cell cultures. Cell 2: 55-61, 1974.

660. Tronick, S.R., Stephenson, J.R., and Aaronson, S.A.: Immunological properties of two polypeptides of Mason-Pfizer monkey virus. J Virol 14: 125-132, July 1974.

661. Tronick, S.R., Stephenson, J.R., Aaronson, S. and Kawakami, T.G.: Antigenic characterization of type C RNA virus isolates of gibbon apes. J Virol 15(1): 115-120, Jan. 1975.

662. Tsoi, M.S., Weiden, P.L. and Storb, R.: Lymphocyte reactivity to autochthonous tumor cells in dogs with spontaneous malignancies. Cell Immunol 13: 431-439, 1974.

663. Tsuchida, N., Gilden, R.V., and Hatanaka, M.: Sarcoma virus related RNA sequences in normal rat cells. Proc Natl Acad Sci USA 71: 4503-4507, Nov. 1974.

664. Tsuchida, N., Gilden, R.V., Hatanaka, M., Freeman, A., and Huebner, R.J.: Type C virus-specific nucleic acid sequences in cultured rat cells. Int J Cancer 15, Jan. 1975.

665. Tsuchida, N. and Green, M.: Intracellular viral RNA species in mouse cells nonproductively transformed by the murine sarcoma virus. J Virol 14(3):587-591, 1974.

666. Tsuchida, N., Long, C., and Hatanaka, M.: Viral RNA of murine sarcoma virus produced by a hamster-mouse somatic cell hybrid. Virology 60: 200-205, July 1974.

667. Tsuchida, N., Shih, M., Gilden, R.V., and Hatanaka, M.: Mutants of non-producer cell lines transformed by murine sarcoma virus. III. Detection and characterization of RNA specific for helper and sarcoma viruses. J Exp Med 140: 218-224, July 1974.

668. Tsuchida, N., Shih, M.S., Gilden, R.V. and Hatanaka, M.: Sarcoma and helper-specific RNA tumor virus subunits in transformed non-producer mouse cells activated to produce virus by treatment with bromodeoxyuridine. J Virol 14: 1262-1267, Nov. 1974.

669. Twardzik, D.R., Simonds, J.R., Portugal, F.: DNA polymerase activities in viro cells infected with herpesvirus saimiri. Biomed 20: 1-5, 1974.

670. Vaidya, A.B., Dion, A.S., Black, M.M. and Moore, D.H.: Detection of murine mammary tumor virus related RNA in human breast tumors. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

671. Vaidya, A.B., Dion, A.S., Black, M.M. and Moore, D.H.: The significance of MuMTV-related RNA detected in some human breast tumors. In: Proc IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

672. Vance, J.C., Sabad, A. and Kersey, J.H.: Influence of cyclic nucleotides on lymphocyte activation by calcium ionophore A23187. Fed Proc 34(3) April 1975, (abstract).

673. Van Nie, R., Hilgers, J. and Verstraeten, A.: Chromosomal and extrachromosomal transmission of mammary tumor virus in the GR mouse strain and the effect on mammary tumorigenesis. In: Proc IXth Mtg on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

674. Varmus, H.E., Guntaka, R.V., Deng, C.T. and Bishop, J.M.: Synthesis, structure, and function of Rous sarcoma virus-specific DNA in permissive and non-permissive cells. In: Cold Spring Harbor Symposium, Vol XXXIX, Tumor Viruses, Cold Spring Harbor Laboratory, New York, April 1975.

675. Varmus, H.E., Guntaka, R.V., Deng, C.T., Domenik, C. and Bishop, J.M.: Synthesis and function of avian sarcoma virus-specific DNA in permissive and non-permissive host cells. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

676. Varmus, H.E., Guntaka, R.V., Fan, W., Heasley, S. and Bishop, J.M.: Synthesis of viral DNA in the cytoplasm of duck embryo fibroblasts and in enucleated cells after infection by avian sarcoma virus. Proc Natl Acad Sci USA 71(10): 3874-3878, Oct. 1974.

677. Varmus, H.E., Heasley, S. and Bishop, J.M.: Use of DNA-DNA annealing to detect new virus-specific DNA sequences in chicken embryo fibroblasts after infection by avian sarcoma virus. J Virol 14(4): 895-903, Oct. 1974.

678. Vecchio, G., Shanmugam, G., Bhaduri, S., Gianni, A.M. and Green, M.: Translation of RNA tumor virus messenger RNA (mRNA) in vivo and in vitro. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 252, (abstract).

679. Verma, I.M., Firtel, R.A., Lodish, H.F. and Baltimore, D.: Synthesis of DNA complementary to cellular slime mold messenger RNA by reverse transcriptase. Biochemistry 13: 3917-3922, Nov. 1974.

680. Verma, I.M., Mason, W.S., Drost, S.D. and Baltimore, D.: DNA polymerase activity from two temperature sensitive mutants of Rous sarcoma virus is thermolabile. Nature 251: 27-31, Sept. 1974.

681. Verstraeten, A., Blankevoort, R., van Nie, R., and Kwa, H.G.: Radioimmunoassay studies on the distribution of antigens common to the mouse mammary tumour virus (MTV) and the nodule inducing virus (NIV) in milk of various strains and hybrids. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

682. Verstraeten, A. and Van Nie, R.: Radioimmunologic evidence for a linkage relationship between the gene controlling MTV-L expression in milk and the albino (c) locus. In: Proc IXth Mtg on Mammary Cancer in Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

683. Vigier, P. and Montagnier, L.: Infectious DNA recovered from avian tumor-virus-producing cells. Int J Cancer 15(1): 67-77, Jan. 1975.

684. Vogt, P.K. and Duesberg, P.H.: Elementary aspects of RNA tumor virus genetics. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

685. Vogt, P.K. and Harris, P.: Use of plastic bags to maintain a humidified atmosphere for animal cell cultures. Appl Microbiol 27: 618-619, 1974.

686. Vogt, P.K., Wyke, J.A., Weiss, R.A., Friis, R.R., Katz, E. and Linial, M.: Avian RNA tumor viruses: Mutants markers and genotypic mixing. In: Molecular Studies in Viral Neoplasia, The Williams and Wilkins Co., Baltimore, 1974, pp 190-205.

687. Wahl, D.V., Chapman, W.H., Hellström, I., Hellström, K.E.: Transplantation immunity to individually unique antigens of chemically induced bladder tumors in mice. Int J Cancer 14: 114-121, 1974.

688. Wallen, W.C., Rabin, H., Neubauer, R.H. and Cicmanec, J.L.: Depression in lymphocyte response to general mitogens by owl monkeys infected with herpesvirus saimiri. J Natl Cancer Inst 54, 1975.

689. Wang, L.H. and Duesberg, P.: Properties and location of Poly(A) in Rous sarcoma virus RNA. J Virol 14(6): 1515-1529, Dec. 1974.

690. Waters, L.C., Mullin, B.C., Ho, T. and Yang, W.K.: In vitro association of unique species of cellular 4S RNA with 35S RNA of RNA tumor viruses. Biochem Biophys Res Comm 60(2): 489-497, Sept. 1974.

691. Waters, L.C. and Yang, W.K.: Comparative biochemical properties of RNA-directed DNA polymerases from Rauscher murine leukemia virus and avian myeloblastosis virus. Cancer Res 34: 2585-2593, Oct. 1974.

692. Weiden, P., Storb, R., Kolb, H.J., Ochs, H.D., Graham, T.C., Tsoi, M.S., Schroeder, M.L. and Thomas, E.D.: Immune reactivity in dogs with spontaneous malignancy. J Natl Cancer Inst 53: 1049-1056, Oct. 1974.

693. Whitmire, C.E., Demoise, C.F., Kouri, R.E.: The role of the host in the development of in vivo models for carcinogenesis studies. In: Symp on Experimental Respiratory Carcinogenesis and Bioassays. (J.F. Parks and E. Karbe, eds.) Springer-Verlag, 1974, pp 20-47.

694. Whitten, H.D., Lamon, E.W. and Andersson, B.: IgM complex receptors on T and B lymphocytes. 7th Annual Miami Winter Symp, Vols 9 & 10, Miami, Fla., Jan. 1975, (abstract).

695. Williams, E.H., Day, N.E., and Geser, A.: Seasonal variation in onset of Burkitt's lymphoma in the West Nile district of Uganda. Lancet, ii: 19-22, 1974.

696. Williams, E.H., and De Thé, G.: Familial aggregation of nasopharyngeal carcinoma (NPC), letter, Lancet ii: 295, 1974.

697. Wright, J., Falk, L.A. and Deinhardt, F.: Induction of Epstein-Barr virus nuclear antigen (EBNA) in human cord blood lymphocytes. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

698. Wright, J., Falk, L.A. and Deinhardt, F.: Interferon production by simian lymphoblastoid cell lines. J Natl Cancer Inst 53(1): 271-275, July 1974.

699. Wright, P.J. and Di Mayorca, G.: Virion polypeptide composition of the human papovavirus, BK. Abstracts Annual Mtg Am Soc Microbiology, April 27-May 2, 1975, New York, N.Y.

700. Wright, P.W., Bernstein, I.D., Hamilton, B., Gluckman, J.C., and Hellström, K.E.: Cell-mediated reactivity and serum blocking activity in tolerant rats. Studies on ten rats operationally tolerant to skin allografts, using several independent assays. Transplantation 18: 46-55, 1974.

701. Wright, P.W., Hargreaves, R.E., Bernstein, I.D. and Hellström, I.: Fractionation of sera from operationally tolerant rats by DEAE cellulose chromatography: Evidence that serum blocking factors are associated with IgG. J Immunol 112: 1267-1270, 1974.

702. Wu, A.M., Cetta, A., Sarngadharan, M.G. and Gallo, R.C.: Two forms of viral DNA polymerases (R-MuLV) with different biochemical and immunological properties. Fed Proc 34(3) April 1975, (abstract).

703. Wu, A.M. and Gallo, R.C.: Interaction between murine type C virus RNA directed DNA polymerases and rifamycin derivatives. Biochem Biophys Acta 340: 419-436, 1974.

704. Wu, A.M. and Gallo, R.C.: Life cycles of RNA oncogenic viruses. In: Symp on Modern Trends in Human Leukemia: Biological, Biochemical and Virological Aspects, 1973, (R. Neth, ed.) Wilsede, West Germany, 1974, pp 148-156.

705. Wu, A.M. and Gallo, R.C.: Selective compounds that affect the replication cycle for RNA tumor viruses. Conf on Tumor Viruses-Human Tumors, 1974, p 83, (abstract).

706. Wu, A.M., Paran, M., Reitz, M. and Gallo, R.C.: Stimulation of murine type C RNA tumor virus production by glucogenic corticosteroid and their mechanism of action. Panel presentation on Molecular Biology of Viral Oncogenesis. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, pp 370, (abstract).

707. Wu, A.M., Prival, J., Paran, M. and Gallo, R.C.: Hemopoietic stem cells and leukemia. In: Symp on Modern Trends in Human Leukemia: Biological, Biochemical and Virological Aspects, 1973, (R. Neth, ed.), Wilsede, West Germany, 1974, pp 60-73.

708. Wu, A.M., Prival, J., Paran, M. and Gallo, R.C.: On the heterogeneity of colony stimulating factors prepared from human peripheral blood in culture in the presence of mitogen. 17th Annual Mtg of Am Soc Hematol, Atlanta, Ga., Dec. 1974, p 78, (abstract).

709. Wu, A.M., Reitz, M.S., Paran, M. and Gallo, R.C.: On the mechanism of stimulation of murine type C RNA tumor virus production by glucocorticoids--Post-transcriptional effects. J Virol 14: 802-812, 1974.

710. Yamashita, T. and Green, M.: Adenovirus DNA replication I. Requirement for protein synthesis and isolation of nuclear membrane fractions containing newly synthesized viral DNA and proteins. J Virol 14(3): 412-420, 1974.

711. Yang, C.S. and Hewetson, J.F.: Cytotoxic antibodies to cultured lymphoblasts unrelated to Epstein-Barr virus. Int J Cancer 14: 530-538, 1974.

712. Yang, W.K., Ihle, J.N., Koh, C.K., Ho, T. and Waters, L.C.: Chromatographic, metabolic and hybridization characteristics of 4S RNA's from RNA tumor viruses. Proc XIth Int Cancer Congress, Florence, Italy, Oct. 1974, (abstract).

713. Yang, W.K., Tennant, R.W., Schluter, B., Myer, F.E. and Brown, A.: Biochemical characterization of Fv-1 allele cell extracts inhibiting mouse leukemia virus infection. Proc Am Assoc for Cancer Res & Am Soc Clinical Oncology, May 7-11, 1975, San Diego, California, (abstract).

714. Yaniv, A., Ohno, T., Kacian, D., Colcher, D., Witkin, S., Schlom, J., and Spiegelman, S.: Seriological analysis of reverse transcriptase of the Mason-Pfizer monkey virus. Virology 59: 335-338, 1974.

715. Yata, J., Gatti, R.A., Klein, G., Good, R.A., and Tsukimoto, I.: Lymphocyte subpopulations in immunodeficiency disorders. I. Human thymus-lymphoid tissue antigens. Clin Immunol Immunopathol 2: 519-525, 1974.

716. Yeh, J., Ahmed, M., Lyles, J.M., Larson, D.L. and Mayyasi, S.: Competition radioimmunoassay for Mason-Pfizer virus: Comparison with new isolates. Proc IXth Mtg on Mammary Cancer in Experimental Animals and Man, Pisa, Italy, Oct. 1974, (abstract).

717. Yeh, J., Ahmed, M., Lyles, J., Larson, D., and Mayyasi, S.: Competition radioimmunoassay for Mason-Pfizer monkey virus: Comparison with recent isolates. Int J Cancer 15, 1975.

718. Yoshiki, T., Mellors, R.C., Strand, M. and August, J.T.: Structural proteins of murine leukemia virus and the immune complex glomerulonephritic of New Zealand mice and mice of other strains: Involvement of viral envelope glycoprotein, gp69/71, in the pathogenesis. J Exp Med 140: 1011, 1974.

719. Young, H.A., Scolnick, E.M. and Parks, W.P.: Glucocorticoids and breast cancer viruses in tissue culture: Program of the Breast Cancer Task Force. Div Cancer Biol and Diag, NCI, NIH, p 43, Feb. 1975, (abstract).

720. Zeve, V.H., Gonda, M.A., Lebiedzik, J.: Application of an automated particle analysis system to the quantitation of virus particles. J Natl Cancer Inst 53: 1099-1122, Oct. 1974.

721. Aaronson, S.A. and Schlom, J.: The search for RNA tumor viruses in human cancer. In: Progress in Clinical Cancer (I.M. Ariel, ed.) Grune & Stratton, Inc., New York, 1975 (In Press).

722. Aaronson, S.A. and Stephenson, J.R.: Viruses and the etiology of cancer. In: Clinical Concepts in Virology. (W.L. Drew, ed.) Medcom Press (In Press).

723. Aaslestad, H.G., Twardzik, D.R., Armstrong, G.R., et al: Partial purification and biochemical characterization of herpesvirus saimiri. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

724. Adams, A. and Lindahl, T.: Epstein-Barr virus genomes with properties of circular DNA molecules in carrier cells. Proc Natl Acad Sci USA (In Press).

725. Adams, A. and Lindahl, T.: Intracellular forms of EBV DNA in Raji cells. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

726. Aisenberg, A.C.: Immunological reactions in lymphomas and leukemias. In: Scientific Foundations of Oncology (Prof. T. Symington, ed.) William Heinemann Medical Books, Ltd., London (In Press)

727. Aisenberg, A.C. and Long, J.: Lymphocyte surface characteristics in malignant lymphoma. Am J Med (In Press).

728. Aisenberg, A.C. and Qazi, R.: Abdominal involvement at the onset of Hodgkin's disease. Am J Med (In Press).

729. Allen, P.T., Bowen, J.M., Newton, W.A., East, J.L., Georgiades, J., Muruyama, K., Priori, E.S., Miller, M.F., Nash, M.A., Myers, B. and Dmochowski, L.: Molecular studies of cells derived from human solid tumors. In: Fundamental Aspects of Neoplasia, (A.A. Gottlieb, ed.) Springer-Verlag, New York, 1975 (In Press).

730. Allen, P.T., Bowen, J.M., Newton, W.A., East, J.L., Georgiades, J., Maruyama, K., Priori, E.S., Miller, M.F., Nash, M.A., Myers, B. and Dmochowski, L.: Tumor associated changes in nucleolar antigens. In: Immunological Aspects of Neoplasia, Proc 26th Annual Symp on Fundamental Cancer Res, Univ of Texas, M.D. Anderson Hosp. and Tumor Inst., Houston, Texas, March 1973 (In Press).

731. Altenburg, B. and Steiner, S.: Mechanism of cell agglutination by concanavalin A: Possible role of restricted movement of binding sites. Exp Cell Res (In Press).

732. Ambrose, K.R. and Coggin, J.H., Jr.: Horizontally transmissable hamster lymphosarcoma. J Natl Cancer Inst 1974 (In Press).

733. Anderson, R., Kersey, J.H., Coccia, P.F., Nesbit, M.E., Cervenka, J., Yunis, E. and Krivit, W.: T lymphocyte depletion in familial leukemia. Soc Pediatric Res (abstract) (In Press).

734. Anzai, T., Dreesman, G.R., Courtney, R.J., Adam, E., Rawls, W.E. and Benyesh-Melnick, M.: Antibody to herpes simplex virus type 2 induced nonstructural proteins in women with cervical cancer and in control groups. J Natl Cancer Inst (In Press).

735. Aoki, T. and Sibal, L.R.: Surface antigens associated with type C RNA viruses. Excerpta Med (In Press).

736. Arnold, J., Soule, H.D. and Russo, J.: Fine structure of a human mammary carcinoma cell line, MCF-7. Proc Tissue Culture Assoc (In Press).

737. Arnold, J., Soule, H.D. and Russo, J.: Fine structure of a murine mammary carcinoma cell line. In Vitro (In Press).

738. August, J.T. and Strand, M.: The major envelope glycopeptide of type C RNA tumor viruses: Purification properties and expression. Proc Symp on Biology of Oncogenic Viruses of Animals and Man, Moscow, 1974 (In Press).

739. Auld, D.S., Kawaguchi, H., Livingston, D.M. and Vallee, B.L.: Zinc reverse transcriptases from mammalian RNA type C viruses. Biochem Biophys Res Commun (In Press).

740. Aupoix, M.C. and Vigier, P.: Evidence for a host cell surface antigen on the envelope of avian tumor viruses. J Gen Virol (In Press).

741. Aurelian, L.: Possible role of herpesvirus hominis type 2 in human cervical cancer. Year Book of Cancer 1974, (abstract) (In Press).

742. Aurelian, L., Cornich, J.D. and Smith, M.F.: Characterization of the specific antibody reacting in the tumor-specific HSV type 2 induced antigen in cervical cancer patients. IARC/WHO (In Press).

743. Baldwin, C.L., Lemp, J.F. and Barbeito, M.S.: Biohazards assessment in large scale zonal centrifugation. Appl Microbiol (In Press).

744. Barahona, H., Garcia, F.G., Melendez, L.V. and Ingalls, J.K.: Isolation and characterization of a lymphocyte associated foamy virus from a red uakari monkey (Cacajao rubicundus). J Med Primatol (In Press).

745. Barahona, H., Melendez, L.V. and Melnick, J.L.: A compendium of herpesviruses isolated from non-human primates. Intervirology (In Press).

746. Barlati, S., Kryceve, C. and Vigier, P.: Different stages of transformation of temperature-sensitive Rous cells evidenced by methylene blue staining. Intervirology (In Press).

747. Baron, S., Johnson, H.M., Smith, B.G. and Gazdar, A.F.: Studies of relationship between interferon, antibody and tumor inhibition and enhancement. J Natl Cancer Inst (In Press).

748. Beard, J.W. and Beard, D.: Oncogenicity of avian tumor viruses. In: The Biology of Tumor Viruses, Proc 34th Ann Biol Coll, Corvallis, Oregon, April 1973, (G. Beaudreau, ed.) Oregon State University Press, Corvallis, Oregon (In Press).

749. Becker, L.E., Narayan, O. and Johnson, R.T.: Demonstration of papovavirus tumor antigen in brain tumors. J Neuropathol Exp Neurol (abstract) (In Press).

750. Becker, Y. and Asher, Y.: In vitro synthesis of herpes simplex viral DNA in isolated nuclei. In: Studies on Virus Replication, European Commission, Brussels, 1974 (In Press).

751. Becker, Y., Shlomai, J., Kamincik, Y., Asher, Y. and Ben Zeev, A.: Studies on nuclei from cells infected with herpesviruses (HSV and EB). In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

752. Becker, Y., Weinberg, E. and Cohen, Y.: Studies on the state of EBV DNA synthesis in Burkitt lymphoblasts. In: Int Workshop on Production, Concentration and Purification of Epstein-Barr Viruses and Viral Antigens, NCI, FCRC, Frederick, Md., Feb. 10-12, 1975 (F.V. Ablashi, ed.) 1975 (In Press).

753. Becker, Y., Weinberg, E., Cohen, Y. and Klein, G.: Studies on Epstein-Barr virus specified proteins in Burkitt lymphoblasts. In: Int Workshop on Production, Concentration and Purification of Epstein-Barr Viruses and Viral Antigens, NCI, FCRC, Frederick, Md., Feb. 10-12, 1975 (F.V. Ablashi, ed.) 1975 (In Press).

754. Bekesi, J.G.: Immunotherapy and immunotherapy with Vibrio cholerai neuraminidase-treated tumor cells in man and experimental animals. In: Conflicts in Childhood Cancer (L. Sinks, ed.) (In Press).

755. Bekesi, J.G., Roboz, J.P. and Holland, J.F.: Immunotherapy with neuraminidase-treated murine leukemia cells after cytoreductive therapy in leukemic mice. In: Natl Cancer Inst Monograph: Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias (M.A. Chirigos, ed.) 1975 (In Press).

756. Bekesi, J.G., Roboz, J.P. and Holland, J.F.: Specific immunoprotection and kinetics of humoral and cell-mediated immunity in DBA/2 mice immunized with neuraminidase-treated leukemia L 1210 cells. J Natl Cancer Inst (In Press).

757. Bellevue, R., Dosik, H., Spergel, G., Gussoff, B.D.: Pseudohyperkalemia and extreme leukocytosis. J Lab and Clin Med (In Press).

758. Benveniste, R.E., Sherr, C.J., Lieber, M.M., Callahan, R. and Todaro, G.J.: Evolution of primate type C viral genes. In: Fundamental Aspects of Neoplasia, (A.A. Gottlieb, ed.) Springer-Verlag, New York, 1975 (In Press).

759. Bétuel, H., Cammoun, N., Ellouz, R., Day, N.E., and De Thé, G.: The relationship between nasopharyngeal carcinoma and the HL-A system among Tunisians. IARC, Paris (In Press).

760. Black, M.M.: Immunology of breast cancer: Clinical implications. In: Progress in Clinical Cancer, (I. Ariel, ed.) Grune and Stratton, Inc., New York (In Press).

761. Blaineau, C., Kishida, T., Sallé, M. and Périés, J.: Inhibitory effect of interferon preparations on the induction of endogenous mouse type C viruses by Iododeoxyuridine (IudR). In: Research on Cancer, Hematology, Microbiology, IRCS (In Press).

762. Bolognesi, D.P.: Oncogenic RNA tumor viruses and the host cell membrane. In: Cell Membranes and Viral Envelopes (H.A. Blough and J.M. Tiffany, eds.), Academic Press, London, (In Press).

763. Bolognesi, D.P., Ishizaki, R., Hüper, G., Vanaman, T.C. and Smith, R.E.: Immunological properties of avian oncornavirus polypeptides. Virology (In Press).

764. Bonkowsky, H.L., Bloomer, J.R., Mahoney, M.J., and Ebert, P.S.: Heme synthesis in protoporphyria: Studies in human liver and cultured skin fibroblasts. First Int Porphyrin Meeting, 1975, (abstract). (In Press).

765. Bouillant, A.M.P., Greig, A.S., Lieber, M.M. and Todaro, G.J.: Type C virus production by a continuous line of pig oviduct cells (PFT). J Gen Virol (In Press).

766. Bourne, H.R., Coffino, P. and Tomkins, G.M.: Selection of a variant lymphoma cell deficient in adenylate cyclase. Science (In Press).

767. Bourne, H.R., Coffino, P. and Tomkins, G.M.: Somatic genetic analysis of cyclic AMP action: Characterization of unresponsive mutants. J Cell Physiol (In Press).

768. Boyd, A.L., Orme, T.W. and Boone, C.W.: HSV-2 transformation in a murine system. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

769. Boyd, A.L., Orme, T.W. and Boone, C.W.: Transformation of mouse cells with HSV-2. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

770. Brown, T.D.K., Ernberg, I., and Klein, G.: Studies of Epstein-Barr virus (EBV)-associated nuclear antigen. I. Assay in human lymphoblastoid cell lines by direct and indirect determination of ^{125}I-IgG binding. Int J Cancer (In Press).

771. Brown, T.D.K., Klein, G.: Detection of Epstein-Barr virus (EBV)-associated nuclear antigen in frozen and thawed cells and subcellular fractions of human LCL's by means of an ^{125}I-IgG binding assay. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

772. Buell, P., Henderson, B.E., Louie, E., Menck, H.R., Henle, W. and Pike, M.C.: Selected observations on the epidemiology of carcinoma of the nasopharynx and other pharyngeal cancers. Am J Epidemiol (In Press).

773. Byers, V.S., Levin, A.S., Hackett, A.J. and Fudenberg, H.H.: Tumor specific cell mediated immunity in household contacts of cancer patients. J Clin Invest (In Press).

774. Calafat, J., Hageman, Ph.D., Hilgers, J., Verbeet, M., and Nusse, R.: Distribution of MTV antigens and Thy.1.2 as studied by electronmicroscopy. Symp Borstkanker en RNA Tumorvirussen, 16e Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract) (In Press).

775. Caldwell, G.G. and Heath, C.W., Jr.: Case clustering in cancer. Southern Med J (In Press).

776. Callahan, R., Lieber, M.M. and Todaro, G.J.: Nucleic acid homology of murine xenotropic type C viruses. J Virol (In Press).

777. Cameron, K.R.: Animal viruses, radiation, repair mechanisms and cancer. In: Molecular Mechanisms for the Repair of DNA, Proc ICN-UCLA Winter Conf, Squaw Valley, Calif., 1974 (In Press).

778. Carrico, R.J., Beagle, R., Gomex, M. and Deinhardt, F.: A radioimmunoassay specific for the antigenic determinants exposed by pepsin digestion of human immunoglobulin G. Immunochemistry (In Press).

779. Chan, S.H., Chew, T.S., Yap, J. and Simons, M.J.: In vitro assay of lymphocyte-mediated immunity using ^{75}Selenium-methionine: I. Lymphocyte activation by phytohaemmagglutinin (PHA) in macro cultures. IARC, Paris (In Press).

780. Chan, S.H., Chew, T.S. and Simons, M.J.: In vitro assay of lymphocyte-mediated immunity using ^{75}Selenium-methionine: II. Lymphocyte activation by phytohaemmagglutinin (PHA) in micro cultures. IARC, Paris (In Press).

781. Chan, S.H., Simons, M.J., Chew, T.S., De Thé, G. and Shanmugaratnam, K.: Immunological aspects of nasopharyngeal carcinoma (NPC): I. In vitro lymphocyte hyporesponsiveness stimulation with phytohaemmagglutinin (PHA). IARC, Paris (In Press).

782. Chen, P.K., Kersey, J.H., Clawson, C.C. and Yunis, E.J.: Thymic aplasia with possible graft versus host reaction. J Pediatr (In Press).

783. Chen, P.L., Hutchison, D.J., Sarkar, N.H., Kramarsky, G. and Moore, D.H.: Identification of the virions in the in vitro L1210(V) leukemia cell lines by morphological, virological and immunological techniques. Cancer Res (In Press).

784. Coffino, P., Bourne, H.R. and Tomkins, G.M.: Somatic genetic analysis of cyclic AMP action: Selection of unresponsive mutants. J Cell Physiol (In Press).

785. Coffino, P., Gray, J.W. and Tomkins, G.M.: Cyclic AMP, a nonessential regulator of the cell cycle. Proc Natl Acad Sci USA (In Press).

786. Cohen, J., and Lenoir, G.: Comparative morphology and ultrastructure of four fish rhabdoviruses. Ann Rech Vet, 1974 (In Press).

787. Colcher, D., Spiegelman, S. and Schlom, J.: Sequence homology between the RNA of Mason-Pfizer monkey virus and the RNA of human malignant breast tumors. Proc Natl Acad Sci USA (In Press).

788. Dalton, A.J.: Microvesicles and vesicles of multivesicular bodies vs. "virus-like" particles. J Natl Cancer Inst (In Press).

789. Daniel, M.D., Hunt, R.D., DuBose, D., Silva, D. and Melendez, L.V.: Induction of Herpesvirus saimiri lymphoma in New Zealand white rabbits inoculated intravenously. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

790. Davis, J., Gilden, R.V. and Oroszlan, S.: An antigenically related tryptic polypeptide from several mammalian type C RNA virus p30s. Intervirology (In Press).

791. Deinhardt, F.: Virus-induced lymphproliferative disease in nonhuman primates. Br J Cancer (In Press).

792. Deinhardt, F. Zur Virusätiologie von Krebskrankheiten (Onkogene Viren). Der Internist (In Press).

793. Deinhardt, F. and Deinhardt, J.B.: Oncogenic viruses in primates. Proc Symp on Biology of Oncogenic Viruses of Animals and Man, Moscow, 1974. (In Press).

794. Deinhardt, F., Falk, L., Wolfe, L., Paciga, J. and Johnson, D.: Responses of marmosets to experimental infection with Epstein-Barr virus (EBV). In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

795. Desgranges, C., Wolfe, H., Zur Hausen, H., and De Thé, G.: Further studies on the detection of the Epstein-Barr viral DNA on nasopharyngeal carcinoma biopsies from different parts of the world. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

796. De Thé, G., Ablashi, D.V., Day, N.E., Ho, J.H.C., Macario, A.J.L., Pearson, G. and Sohier, R.: Nasopharyngeal carcinoma. IX. Antibodies to EBNA and correlations with other EBV reactivities. Int J Cancer (In Press).

797. De Thé, G., Day, N.E., Geser, A., Ho, J.H.C., Lavoué, M.F., Simons, M.J., Sohier, R., Tukei, P., Vonka, V. and Zavadova, H.: Sero-epidemiology of the Epstein-Barr virus: preliminary analysis of an international study. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

798. De Thé, G., Geser, A. and Munoz, N.: Viruses and human neoplasia. In: Scientific Foundations of Oncology (T. Symington and R.L. Carter, eds.) Wm. Heinemann Medical Books, Ltd., London. (In Press).

799. De Thé, G., Ho, J.H.C. and Muir, C.: Nasopharyngeal carcinoma. In: Viral Infections in Man: Epidemiology and Control. (A.S. Evans, ed.) Plenum Corp., N.Y. (In Press).

800. Didier, M.L., Ablashi, D.V., Oie, H.K., Armstrong, G.R., Chu, E. and Rabson, A.: Some biological properties of herpesvirus saimiri (HVS) from chronically infected monolayer and suspension cultures. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

801. Di Salvo, L.H., Guard, H.E. and Hunter, L.: Tissue hydrocarbon burden of mussels as a potential monitor of environmental hydrocarbon insult. Env Sci and Tech (In Press).

802. Dixon, F.J., Croker, B.P., Del Villano, B.C., Jensen, F.C. and Lerner, R.A.: Immunopathologic consequences of murine oncornavirus infection. Proc 8th Miles Int Symp, Baltimore, June 1974 (In Press).

803. Dmochowski, L.: Morphological, biological, immunological, and biochemical studies on bone tumors of animals and man. Proc VI Int Symp on Malignant Bone Tumors, Dusseldorf, Germany, Oct. 1974, Springer Verlag (In Press).

804. Dmochowski, L.: Viruses and human breast cancer. J Breast (In Press).

805. Dmochowski, L. and Bowen, J.M.: Cell membranes and diseases: Genes, viruses, hormones and the immune response - An introduction. Proc Basic Science Research Symp on Cell Membranes and Diseases: Genes, Viruses, Hormones and the Immune Response. Am J Clin Pathol (In Press).

806. Dosik, H. and Todaro, G.J.: Viruses, genes and cancer. In: Cancer Genetics, Chas. C. Thomas Co., Springfield, Ill. (In Press).

807. Drohan, W.N., Shoyab, M., Wall, R. and Baluda, M.A.: Interspersion of sequences in avian myeloblastosis virus RNA that rapidly hybridize with leukemic chicken cell DNA. Virology, March 1975 (In Press).

808. Duesberg, P.H., Beemon, K., Lai, M. and Vogt, P.K.: Recombinants of avian tumor viruses: An analysis of their RNA. In: Virus Research, Proc ICN-UCLA Symp (C.F. Fox, ed.) Academic Press, New York (In Press).

809. Duff, R.: Quantitative transformation of BALB/3T3 cells by UV-irradiated HSV-2. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

810. Duff, R. and Rapp, F.: Quantitative assay for transformation of 3T3 cells by herpes simplex virus type 2. J Virol (In Press).

811. Easton, J.M., Ablashi, D.V., De Thé, G., Levine, P.H., Twardzik, D.R., Aaslestad, D.R. and Armstrong, G.R.: Association of Epstein-Barr virus (EBV) and American Burkitt's lymphoma (AmBL). In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

812. Emanoil-Ravicovitch, R., Robert-Robin, J., Bazilier, M. and Boiron, M.: Murine oncornavirus high-molecular-weight RNA structure: Thermal stepwise dissociation of 70S murine leukemia-sarcoma virus to subunits and low-molecular-weight associated RNAs. J Virol 15(4) (In Press).

813. Ernberg, I.: Studies on Epstein-Barr virus associated antigens. Ann N Y Acad Sci (In Press).

814. Ernberg, I., Brown, T.D.K.: Studies on the Epstein-Barr virus (EBV)-associated early antigen (EA) with I^{125} labeled IgG. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

815. Falk, L., Wolfe, L. and Deinhardt, F.: Transformation in vitro with herpesvirus ateles (HVA). In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

816. Fowler, A.K., Kouttab, N.M., Kind, P.D., Strickland, J.E. and Hellman, A.: Oncornaviral protein modulation in mouse uterine tissue by estrogen. Proc Soc Exp Biol Med (In Press).

817. Fox, R.R., Diwan, B.A. and Meier, H.: Transplacental induction of primary renal tumors in rabbits treated with ethylnitrosourea. J Natl Cancer Inst (In Press).

818. Frankel, J.W.: Experimental models for DNA-RNA virus interaction: A brief review. Cancer Res (In Press).

819. Frankel, J.W., Smith, M.E., Campbell, W.F., Mitchen, J.R. and Groupe, V.: Experimental evidence for influence of type C viruses on Marek's disease herpesvirus associated oncogenesis. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

820. Friis, R.R., Mason, W.S., Chen, Y.C., Halpern, M.S.: A replication defective mutant of Rous sarcoma virus which fails to make a functional reverse transcriptase. Virology (In Press).

821. Gardner, M.A., Henderson, B.E., Estes, J.D., Rongey, R.W., Casagrande, J., Pike, M. and Huebner, R.J.: The epidemiology and virology of naturally occurring cancer in wild mice as a possible model for human cancer. Cancer Res (In Press).

822. Gerard, G.F., and Grandgenett, D.P.: Purification and characterization of the DNA polymerase and ribonuclease H activities in Moloney murine sarcoma-leukemia virus. J Virol (In Press).

823. Gilden, R.V., Long, C.W., Hanson, M., Toni, R., Charman, H.P., Miller, J.M. and Van der Maaten, M.J.: Partial characterization of bovine leukemia virus. J Gen Virol (In Press).

824. Gillette, R.W.: Homing of labeled lymphoid cells in athymic mice: Evidence for additional immunologic defects. Cell Immunol (In Press).

825. Glaser, R.: Expression and regulation of the Epstein-Barr virus early antigen component(s) in Burkitt-epithelial hybrid cells. Proc 1st Joint Int Workshop on Production, Concentration and Purification of Epstein-Barr Virus Viral Antigens, Feb. 1975, Frederick, Maryland. (In Press).

826. Glaser, R., Decker, B. and Rapp, F.: Oncogenic properties of human viruses. In Vitro (In Press).

827. Glaser, R., Nonoyama, M., Shows, T.B., Henle, G. and Henle, W.: Epstein-Barr virus: Studies on the association of virus genome with human chromosomes in hybrid cells. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

828. Glaser, R. and Rapp, F.: Biological properties of the Epstein-Barr virus and its possible role in human malignancy. In: Progress in Medical Virology (F. Rapp, ed.) S. Karger AG, Basel (In Press).

829. Glaser, R. and Rapp, F.: Control of the Epstein-Barr virus genome by mammalian cells. In: Fundamental Aspects of Neoplasia, (A.A. Gottlieb, ed.) Springer-Verlag, New York, 1975 (In Press).

830. Glaser, R., Zimmerman, J., St. Jeor, S. and Rapp, F.: Demonstration of a cellular inhibitor of Epstein-Barr and cytomegalovirus synthesis. Virology (In Press).

831. Gluckman, E., Schroeder, M.L., Storb, R., Goselink, H , Johnson, S., Graham, T.C., Pretorius, G. and Thomas, E.D.: One-way non-stimulation of mixed leukocyte culture in dog families. Transplantation (In Press).

832. Gluckman, E., Storb, R. and Thomas, E.D.: Traitement des aplasies medullaires et des leucemies aigues par la greffe de moelle osseus allogenique. Progress recent. Actual Hematologiques (In Press).

833. Goodheart, C.R. and Miyagi, M.: Studies of DNA of herpesvirus saimiri. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

834. Grandgenett, D.P., and Rho, H.M.: Binding proteins of avian myeloblastotis virus DNA polymerase to nucleic acid affinity columns. J Virol (In Press).

835. Greenberger, J.S., Rosenthal, D.S., Aaronson, S.A. and Moloney, W.C.: Acute myelogenous leukemia of the Wistar/Furth rat: Establishment of a continuous tissue culture line producing lysozyme in vitro and in vivo. Blood (In Press).

836. Grose, C., Henle, W. and Henle, G.: Primary Epstein-Barr virus infections in acute neurologic diseases. N Engl J Med (In Press).

837. Guntaka, R.V., Bishop, J.M. and Varmus, H.E.: Synthesis and integration of Rous sarcoma virus proviral DNA in duck embryo fibroblast cells. In: Fundamental Aspects of Neoplasia, (A.A. Gottlieb, ed.) Springer-Verlag, New York, 1975 (In Press).

838. Guntaka, R.V., Mahy, B., Bishop, J.M. and Varmus, H.E.: Ethidium bromide inhibits the appearance of closed circular viral DNA and integration of virus-specific DNA in duck cells infected by avian sarcoma virus. Nature (In Press).

839. Gupta, P., Johnson, J. and August, J.T.: Antigenic analysis of major structural proteins of avian leukoviruses. Archives of Biochem and Biophys (In Press).

840. Halpern, M.S., Bolognesi, D.P., Friis, R.R. and Mason, W.S.: Expression of the major viral glycoprotein of avian tumor virus in cells of chf(+) chick embryos. J Virol (In Press).

841. Hampar, B., Tanaka, A., Nonoyama, M. and Derge, J.G.: Sequence of Epstein-Barr virus productive cycle in human lymphoblastoid cells. Proc Symp on Quantitative Biology 39, 1974 (In Press).

842. Hannon, W.H., Ambrose, K.R. and Coggin, J.H., Jr.: Forssman antigen and phase specific fetal antigens: An evaluation of their role in SV40 tumor immunity. Proc Soc Exp Biol Med (In Press).

843. Hartley, J.W. and Rowe, W.P.: Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology (In Press).

844. Hatanaka, M., DelGiudice, R. and Long, C.: Adenine formation from adenosine by mycoplasmas: Adenosine phosphorylase activity. Proc Natl Acad Sci USA (In Press).

845. Heath, C.W., Jr., Caldwell, G.G., Feorino, P.C.: Viruses and other microbes. In persons at high risks of cancer, implications to cancer control. Natl Cancer Inst/Am Cancer Soc Monograph. (In Press).

846. Heberling, R.L. and Kalter, S.S.: Isolation of foamyviruses from baboon tissues. Am J Epidemiol (In Press).

847. Heidel, J.W., Quarles, J.M. and Tennant, R.W.: Computation of DNA synthesis rates from flow microfluorometric histograms. Symp on Biomath and Computer Science in Life Sciences, 1975, (abstract) (In Press).

848. Hellman, A., Fowler, A.K., Strickland, J.E. and Kouttab, N.M.: Oncornaviral information modulation in mouse uterine tissue. 1st Vienna Mtg, Int Assoc Promotion Clinical and Experimental Research in Medicine - Molecular Basis of Malignancy; New Clinical and Therapeutic Evidences (In Press).

849. Hellström, I., Hellström, K.E. and Nishioka, M.: Reactivity to tumor associated antigens detected in mice undergoing liver regeneration. Nature (In Press).

850. Henderson, B.E., Gerkins, V. and Pike, M.C.: Persons at high risk to cancer: An approach to cancer etiology and control. Sexual factors and pregnancy. J Natl Cancer Inst (In Press).

851. Henderson, B.E., Gordon, R.G., Menck, H.R., SooHoo, J., Martin, S. and Pike, M.C.: Lung cancer and air pollution in south-central Los Angeles County. Am J Epidemiol (In Press).

852. Henle, W. and Henle, G.: Host responses to herpes viruses. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

853. Herman, A.C., Green, R.W., Bolognesi, D.P. and Vanaman, T.C.: Comparative chemical properties of avian oncornavirus polypeptides. Virology (In Press).

854. Hilgers, F., Dean, A.G. and De Thé, G.: Elevated immunofluorescence titers to several herpesviruses in Burkitt's lymphoma patients: Are high titers unique? J Natl Cancer Inst (In Press).

855. Hilgers, F., Rosén, A., Lamon, E. and Klein, G.: Detection of EBV determined intracellular antigens in unfixed cells with labeled antibodies. J Natl Cancer Inst (In Press).

856. Hilgers, J.: Genetic aspects of susceptibility to leukemia in mice. Symp Borstkanker en RNA Tumorvirussen, 16e Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract) (In Press).

857. Hilgers, J., Haverman, J., Nusse, R., Van Blitterswijk, W.J., Cleton, F.J., Hageman, Ph.C., Van Nie, R., and Calafat, J.: Immunological, virological and genetical aspects of mammary tumor virus (MTV) induced cell surface antigen: The presence of this antigen as well as the Thy.1.2 antigen on murine mammary gland and tumor cells. J Natl Cancer Inst (In Press).

858. Hilgers, J., Van Blitterswijk, W.J., Bont, W.S., Theuns, G.J., Nusse, R., Haverman, J., and Emmelot, P.: Distribution and antibody-induced redistribution of a MTV-induced and a normal antigen on the surface of mouse leukemic cells. J Natl Cancer Inst (In Press).

859. Hirsch, M.S., Kelly, A.P., Proffitt, M.R. and Black, P.H.: Cell-mediated immunity to antigens associated with endogenous murine C-type leukemia viruses. Science (In Press).

860. Hirsch, M.S., Proffitt, M.R., Black, P.H. and Kelly, A.P.: Immunological activation of viruses and virus activation fof autoimmune responses. In: The Immune System and Infectious Disease, Proc 4th Int Convoc on Immunol, Buffalo, N.Y., 1974 (In Press).

861. Hirsch, M.S., Proffitt, M.R., Ellis, D.A., Black, P.H., Monaco, A.P. and Wood, M.L.: Activation of C-type viruses, during skin graft rejection of the mouse. Interrelationships between immunostimulation and immunosuppression. Int J Cancer (In Press).

862. Hisserich, J.C., Martin, S.P. and Henderson, B.E.: An areawide cancer reporting network. Public Health Reports, 1974 (In Press).

863. Horwitz, C.A., Henle, W., Henle, G. and Schmitz, H.: Clinical evaluation of patients with infectious mononucleosis who developed antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. Am J Med (In Press).

864. Howk, R.S., Anisowicz, A., Silverman, A.Y., Parks, W.P. and Scolnick, E.M.: Distribution of murine type B and type C viral nucleic acid sequences in template active and template inactive chromatin. Cell (In Press).

865. Huebner, R.J.: The endogenous origin and transmission of RNA viral genomes that code for cancer. VIth Int Symp Society for the Conquest of Cancer Diseases, Dusseldorf, Germany, Oct. 1974 (In Press).

866. Hung, C.Y., Perkins, E.H. and Yang, W.K.: Age-related refractoriness of PHA-induced lymphocyte and transformation: I. Comparable sensitivity of spleen cells from young and old mice to culture conditions. Mech Ageing Dev, 1975 (In Press).

867. Hung, C.Y., Perkins, E.H. and Yang, W.K.: Age-related refractoriness of PHA-induced lymphocyte transformation: II. ^{125}I-PHA binding to spleen cells from young and old mice. Mech Ageing Dev, 1975 (In Press).

868. Hunter, L.: The quantitation of environmental hydrocarbons by thin-layer chromatography-A gravimetry densitometry comparison. Env Sci and Tech (In Press).

869. Igel, H.J., Freeman, A.E., Spiewak, J.E. and Kleinfeld, K.L.: Carcinogenesis in vitro. II. Chemical transformation of diploid human cell cultures: A rare event. In Vitro (In Press).

870. Ishizaki, R., Langlois, A.J. and Bolognesi, D.P.: Isolation of two subgroup specific leukemogenic viruses from standard avian myeloblastosis virus. J Virol (In Press).

871. Johansson, B., Holm, G., Mellstedt, H., Henle, W., Henle, G., Söderberg, G., Klein, G. and Killander, D.: Epstein-Barr virus (EBV)-associated antibody patterns in relation to the deficiency of cell-mediated immunity in patients with Hodgkin's disease (HD). In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

872. Jongstra, J., Hilgers, J., Declève, A. and Haas, M.: The occurrence of ecotropic and xenotropic murine leukemia viruses in mice. Symp Borstkanker en RNA Tumorvirussen, 16e Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract) (In Press).

873. Kall, M.A. and Hellström, I.: Specific stimulatory and cytotoxic effect of lymphocytes sensitized in vitro. J Immunol (In Press).

874. Kalter, S.S., Heberling, R.L., Hellman, A., Todaro, G.J. and Panigel, M.: Viruses in the transmission of cancer: C-type particles in the baboon placenta. Proc Roy Soc Med (London) (In Press).

875. Kalter, S.S., Heberling, R.L., Smith, G.C., Panigel, M., Kraemer, D.C., Helmke, R.J. and Hellman, A.: Vertical transmission of C-type viruses: Their presence in baboon follicular oocytes and tubal ova. J Natl Cancer Inst (In Press).

876. Kalter, S.S., Kniker, W.T., Heberling, R.L., Felsburg, P.J., Harvey, J.S. Jr., Macias, E.G., Steele, R.W., Eller, J.J., Rommel, F.A., McCullough, B. and Eichberg, J.: Immunologic aspects of herpesvirus infections in primates. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

877. Kaplan, M.M., Blattner, W.A., Mason, T., Lubiniecki, A.S. and Fraumeni, J.F. Parameters influencing T-antigen expression and transformation in SV40 virus-infected human cells. Fed Proc 34(3) April 1975 (abstract), (In Press).

878. Kaplan, M.M., Giard, D.J., Blattner, W.A., Lubiniecki, A.S. and Fraumeni, J.F., Jr.: An improved method of fixation for immunofluorescent detection of SV40 T-antigen in infected human fibroblasts. Proc Soc Exp Biol Med, March 1975 (In Press).

879. Kersey, J.H. and Booth, G.J.: Cellular recognition and activation within the lymphoid system. Am J Clin Path (In Press).

880. Kersey, J., Nesbit, M., Hallgren, H., Sabad, A., Yunis, E. and Gajl-Peczalska, K.J.: Evidence for origin of childhood acute lymphoblastic leukemias and lymphomas in thymus-derived lymphocytes. Cancer (In Press).

881. Kersey, J.H., Roloff, J., Hokeness, S., Johnson, F.L., Nesbitt, M. and Krivit, W.: Retransplantation of human bone marrow: Two immunosuppressive drug regimens. Transplantation (In Press).

882. Kersey, J.H., Sabad, A., Vance, J.C., White, J.B. and Neely, A.N.: Calcium ionophore as a mitogen: Response of normal human T and B and immunodeficient lymphocytes. Am Soc Clin Invest (abstract). (In Press).

883. Kersey, J. and Spector, B.: Immune deficiency diseases. In: NCI/ACS Workshop on Persons at High Risk for Cancer (J. Fraumeni, ed.) Academic Press, New York (In Press).

884. Kimura, S., Esparza, J., Benyesh-Melnick, M. and Schaffer, P.A.: Enhanced replication of temperature-sensitive mutants of herpes simplex virus type 2 (HSV-2) at the nonpermissive temperature in cells transformed by HSV-2. Intervirology (In Press).

885. Kimura, S., Flannery, V.L., Levy, B. and Schaffer, P.A.: Oncogenic transformation of primary hamster cells by herpes simplex virus type 2 (HSV-2) and an HSV-2 temperature-sensitive mutant. Int J Cancer (In Press).

886. Klein, G., Giovanella, B.C., Lindahl, T., Fialkow, P.J., Singh, S. and Stehlin, J.S.: Direct evidence for the presence of Epstein-Barr virus DNA and nuclear antigen in malignant epithelial cells from patients with anaplastic carcinoma of the nasopharynx. Proc Natl Acad Sci USA (In Press).

887. Klein, G., Sugden, B., Leibold, W. and Menezes, J.: Infection of EBV-genome negative and positive human lymphoblastoid cell lines with biologically different preparations of EBV. Intervirology (In Press).

888. Koment, R.W. and Rapp, F.: Temperature sensitive host range mutants of herpes simplex virus type 2. J Virol (In Press).

889. Koment, R.W. and Rapp, F.: Variation in susceptibility of different cell types to temperature-sensitive host range mutants of herpes simplex virus type 2. Virology (In Press).

890. Kouri, R.W., Kurtz, S., Price, P. and Benedict, W.F.: Studies on the Ara-C-induced transformation of hamster and rat cells in culture. Cancer Res (In Press).

891. Kraemer, D.C., Kalter, S.S. and Moore, G.T.: The establishment of nonhuman primate breeding colonies at the Southwest Foundation for Research and Education. Proc Roy Soc Med (London) (In Press).

892. Kung, P.C., Silverstone, A.E., McCaffrey, R.P. and Baltimore, D.: Murine terminal deoxynucleotidyl transferase: Cellular distribution and response to cortisone. J Exp Med (In Press).

893. Lamon, E.W., Skurzak, H.M., Andersson, B., Whitten, H.D. and Klein, E.: Antibody-dependent lymphocyte cytotoxicity in the murine sarcoma virus system: Activity of IgM and IgG with specificity for MLV determined antigen(s). J Immunol 115(4): April 1975 (In Press).

894. Larsen, C.J., Marty, M., Tavitian, A., Hamelin, R., Périès, J. and Boiron, M.: A search for nucleic acid sequences complementary to a murine oncornaviral genome in Poly (A) rich RNA from human leukemic cells. Proc Natl Acad Sci USA (In Press).

895. Lausch, R.N., Jones, C., Christie, D., Hay, K.A. and Rapp, F.: Spleen cell mediated cytotoxicity of hamster cells transformed by HSV. Evidence for virus specific membrane antigen. J Immunol (In Press).

896. Lausch, R.N., Murasko, D.M., Jones, C. and Hay, K.A.: Immunological studies with hamster cells transformed by cytomegalovirus and herpes simplex virus type 1. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

897. Lausch, R.N., Murasko, D.M., Jones, C. and Hay, K.A.: Presence of virus-associated antigen on the surface of cells transformed by cytomegalovirus and herpes simplex virus. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

898. Lehner, T., Tarro, G., et al: Cell-mediated immunity and antibodies to herpes simplex antigens in leukoplakias and carcinoma in man. Br J Cancer (In Press).

899. Levin, A.C., Massey, R.J., Schauf, V., Deinhardt, F. and Wolter, J.: Use of a ^{51}Cr-release microcytotoxicity assay for the study of lymphocytotoxicity and inhibition of lymphocytotoxicity in human breast cancer. In: Neoplasm Immunity: Theory and Application. (In Press).

900. Levin, A.S., Byers, V.S., Spieler, L.E., Wybran, J., Hackett, A.J. and Fudenberg, H.H.: Osteogenic sarcoma: Immunologic parameters and immunotherapy with tumor specific transfer factor. J Clin Invest (In Press).

901. Levine, P.H., Cho, B.R., Connelly, R.R., Berard, C.W., O'Conor, G.T., Dorfman, R.F., Easton, J.M. and DeVita, V.T.: The American Burkitt lymphoma registry: A progress report. Ann Int Med (In Press).

902. Levine, P.H., Diamond, R.D. and Reisher, J.I.: Antibodies to Epstein-Barr virus (EBV) in patients with cryptococcosis. J Clin Microbiol (In Press).

903. Levine, P.H. and Gravell, M.: The viral etiology of leukemia. In: Modern Problems in Paediatrics (C. Pochedly, ed.) Karger, New York (In Press).

904. Levine, P.H., Herberman, R., McCoy, J., Connelly, R. and Fabrizio, P.: Humoral and cellular immunity to EBV and lymphoid cell line antigens in human lymphoma. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de The and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

905. Lewandowski, L.J., Bolognesi, D.P., Smith, R.E. and Halpern, M.S.: Viral protein synthesis under conditions of glucosamine block in cells transformed by avian sarcoma viruses. Virology (In Press).

906. Li, J., Jerkofsky, M. and Rapp, F.: Demonstration of oncogenic potential of mammalian cells transformed by DNA containing viruses following photodynamic inactivation. Int J Cancer (In Press).

907. Lieber, M.M., Benveniste, R.E., Sherr, C.J. and Todaro, G.J.: Isolation of a type C virus (FS-1) from the European wildcat (Felis sylvestris). Virology (In Press).

908. Lieber, M.M., Sherr, C., Potter, M. and Todaro, G.: Isolation of type C viruses from the Asian feral mouse Mus musculus molossinus. Int J Cancer (In Press).

909. Lieber, M.M., Sherr, C.J. and Todaro, G.J.: Transplantable murine tumors release mouse-tropic and xenotropic type C viruses. Int J Cancer (In Press).

910. Lilly, F., Duran-Reynals, M. and Rowe, W.: Correlation of early murine leukemia virus titer and H-2 type with spontaneous leukemia in mice of the BALB/c x AKR cross: A genetic analysis. J Exp Med (In Press).

911. Lovinger, G., Ling, H.P., Gilden, R.V. and Hatanaka, M.: Effect of UV on RNA directed DNA polymerase activity of murine oncornaviruses. J Virol (In Press).

912. Magrath, I.T., Owor, R., Henley, W. and Olweny, C.: A case of Burkitt's lymphoma occurring in a patient treated for Hodgkin's disease - Epstein-Barr virus antibody titres before and after its development. N Engl J Med (In Press).

913. Martin, S.E. and Martin, W.J.: Anti-tumor antibodies in normal mouse sera. Int J Cancer (In Press).

914. Martin, S.E. and Martin, W.J.: Interspecies brain antigen detected by naturally occurring mouse anti-brain autoantibody. Proc Natl Acad Sci USA (In Press).

915. Martinson, I.M., Kersey, J.H., Dawson, E., Neuburger, C., Anglim, M.A., Veatch, B., Weisensee, M., and Nesbit, M.E.: Home care for the dying child. Am Pediatr Soc (abstract). (In Press).

916. Maruyama, K. and Dmochowski, L.: Surface antigens of RNA virus-induced tumors. In: Immunological Aspects of Neoplasia, Proc 26th Annual Symp on Fundamental Cancer Res, Univ of Texas, M.D. Anderson Hosp and Tumor Inst, Houston, Texas, March 1973 (In Press).

917. McAllister, R.M.: Viruses in human carcinogenesis. Proc Symp on Biology of Oncogenic Viruses of Animals and Man, Moscow, 1974 (In Press).

918. McAllister, R.M., Nelson-Rees, W.A., Peer, M., Laug, W.E., Isaacs, H., Jr., Gilden, R.V., Rongey, R.W. and Gardner, M.B.: Childhood sarcomas and lymphomas: Characterization of new cell lines and search for type C virus. Cancer (In Press).

919. McCaffrey, R., Harrison, T.A., and Baltimore, D.: Chromatographic forms of terminal deoxynucleotidyl transferase in normal and leukemic hematopoietic cells. Clin Res (In Press).

920. McCaffrey, R.P., Harrison, T.A., Parkman, R. and Baltimore, D.: Terminal deoxynucleotidyl transferase activity in human leukemic cells and in normal human thymocytes. N Engl J Med (In Press).

921. McClure, H.M.: Neoplasia of the rhesus monkey. In: The Rhesus Monkey (G.H. Bourne, ed.) Academic Press, New York (In Press).

922. McCoy, J.L., Jerome, L.F., Dean, J.H., Perlin, E., Oldham, R.K., Char, D.H., Cohen, M.H., Felix, E. and Herberman, R.B.: Inhibition of leukocyte migration by tumor-associated antigens in soluble extracts of human malignant melanoma. J Natl Cancer Inst (In Press).

923. Meier, H., Taylor, G.A., Chen, H.W., Heiniger, H.J., Diwan, B.A. and Cherry, M.: Host-gene control of murine type C RNA tumor virus expression and tumorigenesis: Genotypic programming for single and multiple primary tumors. In: Multiple Primary Malignant Tumours, 5th Perugia Quadrennial Int Conf on Cancer, Perugia Univ Med Sch, 28 June-3 July, 1973 (In Press).

924. Melendez, L.V., Cadwallader, J., Jackman, D., Fraser, C.E.O. and Daniel, M.D.: In vitro attenuation of herpesvirus saimiri by serial passage in dog fetal lung (DFL) continuous cultures. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

925. Menck, H.R., Henderson, B.E., Pike, M.C., Martin, S.P., Mack, T. and Soohoo, J.: Cancer incidence in the Mexican American. J Natl Cancer Inst (In Press).

926. Michalides, R., Feldman, S., Spiegelman, S., and Schlom, J.: Comparative studies on the molecular basis for a viral involvement in murine and human mammary adenocarcinoma. Tumor Host Virus Interaction, NATO Adv Study Inst, 1973 (In Press).

927. Michalides, R., Spiegelman, S. and Schlom, J.: Biochemical characterization of putative subviral particulates from human malignant breast tumors. Cancer Res 1975 (In Press).

928. Moore, D.H.: RNA viruses: Mammary tumor virus. In: Cancer: A Comprehensive Treatise. (F.F. Becker, ed.) Plenum Press, New York, N.Y. (In Press).

929. Moore, D.H.: The mammary tumor viruses. In: Fundamental Aspects of Neoplasia (A.A. Gottlieb, ed.) Springer-Verlag, New York, 1975 (In Press).

930. Moore, D.H.: Breast cancer: Its etiology and possible prevention. American Scientist (In Press).

931. Moore, D.H.: Human breast cancer virus. Indian J Cancer (In Press).

932. Munoz, N., Aristizabal, N., Yee, C., Rabson, A., Pearson, G., and De Thé, G.: Antibodies to herpesviruses in patients with cervical cancer and controls. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

933. Nachtigal, M., Albrecht, T., and Rapp, F.: Analysis of chromosomes of Syrian hamster cells transformed with human cytomegalovirus. Intervirology (In Press).

934. Nachtigal, M., Duff, R. and Rapp, F.: Chromosome aberrations in Syrian hamster embryo cells transformed after exposure to ultraviolet irradiated herpes simplex virus type 1 or 2. J Natl Cancer Inst (In Press).

935. Nelson, K., Pollack, S.B., and Hellström, K.E.: Specific anti-tumor responses by cultured immune spleen cells. I. In vitro culture method and initial characterization of factors which block immune cell-mediated cytotoxicity in vitro. Int J Cancer (In Press).

936. Nigida, S.M., Falk, L.A., Wolfe, L.G., Deinhardt, F.W., Long, W.K., and Alford, C.A.: Cytomegalovirus: Latent infection in marmosets experimentally-induced by a human strain (Colburn). Clin Res (In Press).

937. Nonoyama, M.: Detection of tumor virus genomes by nucleic acid hybridization. Int Rev Exp Pathol (In Press).

938. O'Connor, T.E., Schiop-Stansly, P., Sethi, V.S., Hadidi, A. and Okano, P.: Antibiotic inhibition of oncornavirus replication. Proc Gustav Stern Symp on Perspectives in Virology, New York, New York (In Press).

939. Okabe, H., Gilden, R.V., and Hatanaka, M.: Murine sarcoma virus related nucleic acid sequences in a non-transforming virus derived from an interspecies pseudotype sarcoma virus. Int J Cancer (In Press).

940. Oldstone, M.B.A.: Virus neutralization and virus induced immune complex disease: Virus-antibody union resulting in immunoprotection or immunologic injury - Two different sides of the same coin. In: Progress in Medical Virology. (J.L. Melnick, ed.) S. Karger (In Press).

941. Olsen, R.G., Milo, G.E., Schaller, J.P., Mathes, L.E., Heding, L., and Yohn, D.S.: Influence of culture conditions on growth of FL-74 cells and feline oncornavirus cell membrane antigen production. In Vitro (In Press).

942. Olshevsky, U. and Becker, Y.: Synthesis of proteins in herpes simplex virus infected BSC-1 cells. In: Studies on Virus Replication, European Commission, Brussels, 1974 (In Press).

943. Opelz, G., Kiuchi, M., Takasugi, M.: Reactivity of lymphocyte subpopulations in human mixed lymphocyte culture. J Immunogenetics (In Press).

944. Oroszlan, S., Copeland, T., Summers, M.R., Smythers, G. and Gilden, R.V.: Amino acid sequence homology of mammalian type C RNA virus major internal proteins. J Biol Chem (In Press).

945. Oroszlan, S., Summers, M., and Gilden, R.V.; Amino terminal sequence of baboon type C virus p30. Virology (In Press).

946. Osato, T., Nonoyama, M., Mizuno, F., and Yamamoto, K.: Alteration in Epstein-Barr virus-human lymphoid cell interactions by the presence of type C viral genome. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

947. Pagano, J.S., Huang, C.H., Klein, G., De Thé, G., Shanmugaratnam, K., Simons, M.J., and Yang, C.S.: Homology of Epstein-Barr virus DNA in nasopharyngeal carcinomas from Kenya, Tawain, Singapore and Tunis. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, German, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

948. Pal, B.K. and Roy-Burman, P.: Phosphoproteins: Structural components of oncornaviruses. J Virol (In Press).

949. Parks, W.P., Ransom, J.C., Young, H.A. and Scolnick, E.M.: Mammary tumor virus induction by glucocorticoid: Characterization of specific transcriptional regulation. J Biol Chem (In Press).

950. Pearson, G.R., Rabin, H., and Manaker, R.A.: Comparative biology of herpesvirus infections in relation to cancer. Series Virology (USSR) (In Press).

951. Pearson, J.W., Perk, K., Torgersen, J.A., and Chirigos, M.A.: Drug therapy against a transplantable guinea pig leukemia. Cancer Res (In Press).

952. Perk, K., Gazdar, A.F., and Russell, E.R.: Studies on a transplantable murine rhabdomyosarcoma. J Natl Cancer Inst (In Press).

953. Peters, R.L., Donahoe, R.M., and Kelloff, G.J.: An assay in the mouse for delayed type hypersensitivity to murine leukemia virus. J Natl Cancer Inst (In Press).

954. Pike, M.C., Casagrande, J., and Smith, P.G.: Statistical analysis of individually matched case-control studies in epidemiology: Factor under study a discrete variable taking multiple values. Br J Prev Soc Med (In Press).

955. Pike, M.C., Gordon, R.J., Henderson, B.E., Menck, H. and SooHoo, J.: Persons at high risk to cancer: An approach to cancer etiology and control. J Natl Cancer Inst (In Press).

956. Pike, M.C. and Henderson, B.E.: Is cancer contagious? CA (In Press).

957. Pike, M.C. and Smith, P.G.: A note on detecting household aggregation of disease. Biometrics (In Press).

958. Pincus, T. Hartley, J.W., and Rowe, W.P.: A major genetic locus affecting resistance to infection with murine leukemia viruses. IV. Dose-response relationships in Fv-1 sensitive and resistant cell cultures. Virology (In Press).

959. Price, P.J., Suk, W.A., Skeen, P.C., Chirigos, M.A., and Huebner, R.J.: Transforming potential of the anticancer drug adriamycin. Science (In Press).

960. Proffitt, M.R., Hirsch, M.S., Gheridian, B., McKenzie, I.F.C., and Black, P.H.: Immunological mechanisms in the pathogenesis of virus-induced murine leukemia. I. Autoreactivity. Int J Cancer (In Press).

961. Proffitt, M.R., Hirsch, M.S., Gheridian, B., McKenzie, I.F.C. and Black, P.H.: Immunological mechanisms in the pathogenesis of virus-induced murine leukemia. II. Characterization of autoreactive thymocytes. Int J Cancer (In Press).

962. Rand, K., Davis, J., Gilden, R.V., Oroszlan, S., and Long, C.: Fusion inhibition: Bioassay of a type C viral protein. Virology (In Press).

963. Rapp, F. and Duff, R.: The oncogenic potential of herpes simplex virus. Proc Symp on Biology of Oncogenic Viruses of Animals and Man, Moscow, 1974 (In Press).

964. Reed, C.L., Cohen, G.H. and Rapp, F.: Detection of a virus-specific antigen on the surface of herpes simplex virus transformed cells. J Virol (In Press).

965. Reese, J., Reissig, M., Daniel, R.W. and Shah, K.V.: The occurrence of BK virus (BKV) and BKV-specific antibodies in the urine of patients receiving chemotherapy for malignancy. Infect Immun (In Press).

966. Rehacek, J., Fischer, R.G., and Luecke, D.H.: Oncogenic viruses in vertebrates transmitted by hematophagous arthropods. Adv Virus Res, Vol. 20 (In Press).

967. Richardson, L.S., Ting, R.C., Gallo, R.C., and Wu, A.M.: Effect of cordycepin on the replication of type C RNA tumor viruses. Int J Cancer (In Press).

968. Riggs, J.L., McAllister, R.M. and Lennette, E.H.: Immunofluorescent studies on RD-114 virus in cell culture. J Gen Virol (In Press).

969. Ringold, G., Lasfargues, E., Bishop, J.M., and Varmus, H.E.: Production of mouse mammary tumor virus by cultured cells in the absence and presence of hormones: Assay by molecular hybridization. Virology (In Press).

970. Robert-Gero, M., Lawrence, F., and Vigier, P.: Reversible inhibition by methioninyl adenylate of protein synthesis and growth in chick embryo fibroblasts. Biochem Biophys Res Commun (In Press).

971. Robin, M.S., Salzberg, S., and Green, M.: Cytoplasmic synthesis of viral DNA early during infection and cell transformation by the murine sarcoma-leukemia virus. Intervirology (In Press).

972. Rongey, R.W., Abtin, A., Estes, J.D., and Gardner, M.B.: Mammary tumor virus particles in submaxillary gland, seminal vesicle and non-mammary tumors of wild mice. J Natl Cancer Inst (In Press).

973. Rosen, J.M., Woo, S.L.C., and Comstock, J.P.: Regulation of casein messenger RNA during the development of the rat mammary gland. Biochemistry (In Press).

974. Russo, J.P., Furmanski, P. and Rich, M.A.: An ultrastructure study of normal human mammary epithelial cells in culture. Am J Anatomy (In Press).

975. Sadowski, J.M. and Rapp, F.: Inhibition by levamisole of metastases by cells transformed by herpes simplex virus type 1. Proc Soc Exp Biol Med (In Press).

976. Sanford, E.J., Rohner, T.J. and Rapp, F.: Virology of prostate cancer. Proc Natl Prostatic Cancer Project Workshop in Cancer Chemotherapy Reports (In Press).

977. Sarkar, N.H., Manthey, W.J., and Sheffield, J.B.: The morphology of murine oncornaviruses following different methods of preparation for electron microscopy. Cancer Res (In Press).

978. Sarma, P.S., Jain, D.K., Mishra, N.K., Vernon, M.L., Paul, P.S., Pomeroy, B.S.: Isolation and characterization of viruses from natural outbreaks of reticuloendotheliosis in turkeys. J Natl Cancer Inst (In Press).

979. Sarma, P.S., Log, T., Jain, D., Hill, P.R., Huebner, R.J.: Differential host range of viruses of feline leukemia-sarcoma complex. Virology (In Press).

980. Sass, B., Rabstein, L.S., Madison, R., Nims, R.M., Peters, R.L., and Kelloff, G.J.: Incidence of spontaneous neoplasms in 344 SPF Rischer rats throughout the natural lifespan. J Natl Cancer Inst (In Press).

981. Schaffer, P.A.: Temperature-sensitive mutants of herpesviruses. Current Topics in Microbiol Immunol (In Press).

982. Schauf, V., Falk, L., and Deinhardt, F.: Effect of Bacillus Calmette-Guerin immunization in marmoset monkeys infected experimentally with herpesvirus saimiri. J Natl Cancer Inst (In Press).

983. Schauf, V., Massey, R., Deinhardt, F., and Kruse, R.: Immunization of Rous sarcoma virus-inoculated marmosets with BCG and transformed allogeneic cells. J Natl Cancer Inst (In Press).

984. Schlom, J., Colcher, D., Michalides, R., Schochetman, G., Young, J., Spiegelman, S., and Feldman, S.: Biochemical evidence for a viral involvement in human breast cancer. In: Fundamental Aspects of Neoplasia (A.A. Gottlieb, ed.) Springer-Verlag, New York, 1975 (In Press).

985. Schochetman, G. and Schlom, J.: RNA subunit structure of Mason-Pfizer monkey virus. J Virol (In Press).

986. Schroeder, M.L., Gluckman, E., Storb, R. and Thomas, E.D.: One-way non-stimulation of mixed leukocyte culture in canine families. Transplant Proc (In Press).

987. Schroeder, M.L., Storb, R., Graham, T.C. and Weiden, P.L.: Canine radiation chimeras: An attempt to demonstrate serum blocking factors by an in vivo approach. J Immunol (In Press).

988. Schroeder, M.L., Warren, R.P., Storb, R., Goselink, H. and Johnson, S.: Cell-mediated lympholysis in the dog. Transplant Proc (In Press).

989. Sherr, C.J. and Todaro, G.J.: Primate type C virus p30 antigen in human acute leukemia cells. Science (In Press).

990. Sibal, L.R. and Aoki, T.: Immunological control of RNA tumor viruses: Antigenic characterization. Proc Symp on Biology of Oncogenic Viruses of Animals and Man, Moscow, 1974 (In Press).

991. Simons, M.J., Wee, G.B., Chan, S.H., Shanmugaratnam, K., Day, N.E. and De Thé, G.: Immunogenetic aspects of nasopharyngeal carcinoma (NPC). III. HL-A type as a genetic marker of NPC predisposition to test the hypothesis that EBV is an aetiological factor in NPC. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publication, Lyon, France (In Press).

992. Sinkovics, J.G., Györkey, F., Wang, C.-H., Loh, K.K., Romero, J.J., Kay, H.D., and Shullenberger, C.C.: Permanent human lymphoid cell lines: Growth characteristics, morphology and immunology. Proc Vth Int Congress Lymphology, 1975 (In Press).

993. Sinkovics, J.G., Loh, K.K., Thota, H., Kay, H.D., and King, D.: Prospectives for immunotherapy of human sarcomas. XIX Ann Clin Conf Cancer Chemotherapy - Fundamental Concepts and Recent Advances, Houston, Texas, 1975 (In Press).

994. Sinkovics, J.G., Thota, H., Kay, H.D., Loh, K.K., Williams, D.E., Howe, C.D. and Shullenberger, C.C.: Intensification of immune reactions by immunotherapy: Attempts at measuring sarcoma-specific reactions in vitro. In: Neoplasm Immunity: Theory and Application, Chicago Symp Inst Tuberculosis Res, (R.G. Crispen, ed.) Univ of Ill. Schori Press, Evanston, Ill., 1974 (In Press).

995. Sinkovics, J.G., Wang, C.-H., Györkey, F.: Hairy cells in culture. Lancet (In Press).

996. Smith, E.C., Kalter, S.S., Helmke, R.J., Heberling, R.L., Panigel, M., Kraemer, D.C.: A-type particles in placentas of four mouse strains. Proc Soc Exp Biol Med (In Press).

997. Souissi, T., Hilgers, J., Verstraeten, A.A., Kwa, H.G., and Van Nie, R.: Mammary tumor virus expression in genital tract organs of male mice from low and high mammary cancer strains. Symp Borstkanker en RNA Tumorvirussen, 16e Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract)(In Press).

998. Spiegelman, S., Axel, R., Baxt, W., Kufe, D., and Schlom, J.: The molecular genetics of human cancer and its etiologic implications. Genetics (In Press).

999. Spira, G., Estes, M.K., Dreesman, G.R., Butel, J.S. and Rawls, W.E.: Papovavirus structural polypeptides: Comparison of human and rabbit papilloma viruses with simian virus 40. Intervirology (In Press).

1000. Steiner, M., Klein, E., and Klein, G.: Antinuclear reactivity of sera in patients with leukemia and other neoplastic diseases. Clin Immunol Immunopathol (In Press).

1001. Steiner, S., Steiner, M., and Melnick, J.L.: Altered fucolipid patterns in cells transformed by herpes virus type 2. In Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

1002. Storb, R.: The role of marrow transplantation for the treatment of aplastic anemia and acute leukemia. Med Hyg (In Press).

1003. Storb, R., Thomas, E.D., Buckner, C.D., Clift, R.A., Fefer, A., Johnson, F.L. and Neiman, P.E.: Marrow grafting for the treatment of aplastic anemia and acute leukemias. Deutscher Bluttransfusions Kongress (In Press).

1004. Storb, R., Thomas, E.D., Buckner, C.D., Clift, R.A., Johnson, F.L., Fefer, A., Glucksberg, H., Lerner, K.G., Neiman, P.E., Weiden, P.L. and Wright, S.E.: Aplastic anemia (AA) treated by allogeneic marrow grafting. Transplant Proc (In Press).

1005. Storb, R., Weiden, P.L., Schroeder, M.L. and Graham, T.C.: Marrow grafts between canine littermates showing one-way non-stimulation in mixed leukeocyte culture. Transplant Proc (In Press).

1006. Strand, M. and August, J.T.: Structural proteins of mammalian RNA tumor viruses: Relatedness of the interspecies antigen determinants of the major internal protein. J Virol (In Press).

1007. Sugden, B.: Viral RNA in Epstein-Barr virus carrying cells. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

1008. Sumaya, C.V., Henle, W., Henle, G., Smith, M.H.D. and LeBlanc, D.J.: Seroepidemiologic field study of antibodies to Epstein-Barr virus. Antibodies to Epstein-Barr virus. J Infect Dis (In Press).

1009. Svedmyr, E. and Jondal, M.: Presence of cytotoxic effector cells specific for Epstein-Barr virus transformed B cell lines in patients with infectious mononucleosis. Proc Natl Acad Sci USA (In Press).

1010. Tanaka, A. and Nonoyama, M.: Latent genomes of Epstein-Barr virus. Proc 2nd Int Symp on Oncogenesis and Herpesviruses, 1974 (In Press).

1011. Tarro, G.: Analysis and description of procedures used in the study of the relationship of herpes simplex "nonvirion" antigens to certain cancers. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

1012. Tarro, G.: Viral etiopathogenetic perspectives in lung oncology. Arch Monaldi Tisiel Mal App Resp (In Press).

1013. Tarro, G., Battista, A., Di Gioia, M. and Cocchiara, R.: Herpes simplex viruses 1 and 2 "nonvirion" antigens and specific antibodies in immune animals and cancer patients. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

1014. Tarro, G., Battista, A., Sisillo, E. and Smeraglia, R.: Herpes simplex virus genetic markers: Relation to squamous cell carcinomas. Riforma Medica (In Press).

1015. Taylor, J.M., Cordell-Stewart, B., Rohde, W., Goodman, H.M., and Bishop. J.M.: Reassociation of 4S and 5S RNAs with the genome of avian sarcoma virus. Virology (In Press).

1016. Tennant, R.W.: Cellular factors which influence radiation activation and restriction of mouse leukemia viruses. Symp on the Biology of Radiation Carcinogenesis. Gatlinburg, 1975 (abstract)(In Press).

1017. Thomas, E.D., Storb, R., Buckner, C.D., Clift, R.A. and Fefer, A.: Current status of bone marrow transplantation. In: News in Hematology. (A.I. Vorobjev and Y.I. Lorie, eds.) Meditsina, Moscow (In Press).

1018. Thomas, E.D., Storb, R., Clift, R.A., Fefer, A., Johnson, F.L., Neiman, P.E., Lerner, K.G., Glucksberg, H. and Buckner, C.D.: Marrow transplantation. N Engl J.Med (In Press).

1019. Thota, H., Sinkovics, J.G., Carrier, S.K., Romero, J.J. and Kay, H.D.: Cytotoxic lymphocytes. III. Cross-reactions between melanoma and sarcoma cells as expressed by lymphocytes and serum factors of patients with melanoma and sarcoma and of normal donors. Proc IX Int Pigment Cell Conf, 1975 (In Press).

1020. Ti, M., Elguezabel, A., Dosik, H.: Lymphosarcoma of the breast. Am J Med Sci (In Press).

1021. Traul, K.A., and Mayyasi, S.A.: Large scale production of Epstein-Barr virus. In: Int Workshop on Production, Concentration and Purification of Epstein-Barr Viruses and Viral Antigens, NCI, FCRC, Frederick, Md., Feb. 10-12, 1975 (F.V. Ablashi, ed.) 1975. (In Press).

1022. Tronick, S.R., Stephenson, J.R., Verma, I.M., and Aaronson, S.A.: A mutant of murine leukemia virus with a thermolabile reverse transcriptase. In: DNA Synthesis and Its Regulation, ICN-UCLA Conferences in Molecular and Cellular Biology (C.F. Fox, ed.) 1975 (In Press).

1023. Tsoi, M.S., Storb, R., Weiden, P.L., Graham, T.C., Schroeder, M.L. and Thomas, E.D.: Canine marrow transplantation: Are serum blocking factors necessary to maintain the stable chimeric state? J Immunol (In Press).

1024. Tsoi, M.S., Storb, R., Weiden, P.L., Schroeder, M.L. and Thomas, E.D.: Canine marrow transplantation: Do serum blocking factors maintain stable graft-versus-host "tolerance"? Transplant Proc (In Press).

1025. Tsoi, M.S., Weiden, P.L. and Storb, R.: Immunologic reactivity to autochtohonous soluble tumor extracts in dogs with spontaneous malignancies. Reticuloendothel Soc, 1974, (abstract) (In Press).

1026. Tucker, T., Steiner, M.R. and Steiner, S.: Inhibition of cardiolipin synthesis following infection with herpes simplex virus. Intervirology (In Press).

1027. Twardzik, D.R., Aaslestad, H.G., Ablashi, D.V., et al: Comparison of DNA polymerase activities from an American Burkitt's lymphoma cell line and EBV producer and non-producer cells. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974, (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

1028. Van Blitterswijk, W.J., Hilgers, J., Emmelot, P., Kamlag, D., Hilkmann, H.A.M. and Verbeet, M: Release of MTV-induced antigens from the surface of mouse leukemia cells. Symp Borstakanker en RNA Tumorvirussen, 16e Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract) (In Press).

1029. Vance, J.C., Sabad, A., Kersey, J., Goltz, R., Gentry, W., and Cervenka, J.: Lymphocyte abnormalities in the small cell variant of Sezary syndrome. Soc Invest Dermatol (abstract) (In Press).

1030. Van Nie, R., and Verstraeten, A.A.: Genital aspects of the transmission of mammary tumor virus in the GR mouse strain. Symp Borstkanker en RNA Tumorvirussen, 1fe Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract) (In Press).

1031. Verstraeten, A.A., and Van Nie, R.: Genetical aspects of the transmission of the mammary tumor virus (nodule inducing virus) in the C3Hf mouse strain. Symp Borstkanker en RNA Tumorvirussen, 16e Federatieve Vergadering, Utrecht, The Netherlands, April 1975 (abstract) (In Press).

1032. Verstraeten, A.A., Van Nie, R., Kwa, H.G., and Nageman, Ph.C.: Quantitative estimation of mouse mammary tumour virus (MTV) antigens by radioinmunoassay. Int J Cancer (In Press).

1033. Wang, C.H., Sinkovics, J.G., Kay, H.D., Györkey, F. and Shullenberger, C.C.: Growth of permanent lymphoid cell cultures from human source : X Anniversary. Tex Rep Biol Med, 1975 (In Press).

1034. Warren, R.P., Tsoi, M.S., Henderson, B.H., Weiden, P.L. and Storb, R.: Demonstration of tumor-associated antigens in dogs. Transplant Proc (In Press).

1035. Warren, R.P., Tsoi, M.S., Weiden, P.L. and Storb, R.: Production of migration inhibition factor by canine lymphocytes when cultured with autochthonous tumor cells. Reticuloendothel Soc, 1974, (abstract) (In Press).

1036. Weiden, P.L., Storb, R., Kolb, H.J., Graham, T.C., Kao, G. and Thomas, E.D.: Effect of time on sensitization to hemapoietic grafts by preceding blood transfusion. Transplantation (In Press).

1037. Weiden, P.L., Storb, R., Lerner, K.G., Kao, G.F., Graham, T.C. and Thomas, E.D.: Treatment of canine malignancies by 1200 R total body irradiation and autologous marrow grafts. Exp Hematol (In Press).

1038. Wernet, D. and Lilly, F.: Genetic regulation of the antibody response to H-2D^{b} alloantigens in mice. I. Differences in activation of helper T cells in C57BL/10 and BALB/c congenic strains. J Exp Med (In Press).

1039. Wernet, D. and Lilly, F.: Separation of T cell killer function and T cell helper function in B10.A(5R) mice immunized with B10 cells. Transpl Proc (In Press).

1040. Williams, D.E., Romero, J.J., Sinkovics, J.G. and Campos, L.T.: Assessment of sarcoma-specific immune responses of patients during immunotherapy. Texas Med 1975 (In Press).

1041. Wright, J., Falk, L. and Deinhardt, F.: Appearance of Epstein-Barr virus nuclear antigen (EBNA) in human cord blood lymphocytes. In: Oncogenesis and Herpesviruses, Proc 2nd Int Symp on Oncogenesis and Herpesviruses, Nuremberg, Germany, Oct. 1974 (G. de Thé and H. zur Hausen, eds.) IARC Sci Publications, Lyon, France (In Press).

1042. Wright, J., Falk, L. and Deinhardt, F.: Epstein-Barr virus (EBV): Appearance of nuclear antigen in human cord blood lymphocytes. J Clin Res (In Press).

1043. Wright, P.J. and Di Mayorca, G.: Virion polypeptide composition of the human papovavirus, BK: Comparison with simian virus 40 and polyoma virus J. Virology (In Press).

1044. Wright, W.E. and Hayflick, L.: Nuclear control of cellular aging demonstrated by hybridization of anucleate and whole cultured normal human fibroblasts. Exp Cell Res 1975 (In Press).

1045. Wright, W.E. and Hayflick, L.: The regulation of cellular aging by nuclear events in cultured normal human fibroblasts (WI-38). In: Philadelphia Symp on Aging, Plenum Publishing Corp., 1975 (In Press).

1046. Wright, W.E. and Hayflick, L.: The use of biochemical lesions for the selection of human cells with hybrid cytoplasms. Proc Natl Acad Sci USA 1975 (In Press).

1047. Wu, A.M. and Cetta, A.: On the stimulation of viral DNA polymerase activity by nonionic detergent. Biochemistry (In Press).

1048. Yamashita, T., Arens, M. and Green, M.: Adenovirus DNA replication. II. Synthesis of viral DNA in vitro by nuclear membrane fragment from infected KB cell. J Biol Chem (In Press).

1049. Yata, J., Desgranges, C., Nakagawa, T., Favre, M. Cl. and De Thé, G.: Lymphoblastoid transformation and kinetics in the appearance of Epstein-Barr viral nuclear antigen (EBNA) in cord blood cells by Epstein-Barr virus (EBV) infection. Int J Cancer (In Press).

1050. Young, H.A., Scolnick, E.M. and Parks, W.P.: Glucocorticoid-receptor interaction and induction of murine mammary tumor virus. J Biol Chem (In Press).

1051. Young, L.J.T., Cardiff, R.D. and Ashley, R.: Long term primary culture of mouse mammary tumor cells: Production of virus. J Natl Cancer Inst (In Press).

1052. Zajac, B.A. and Ogburn, C.A.: Preparation of rabbit antisera to Epstein-Barr virus D-early antigens. In: Oncogenesis and Herpesviruses, IARC Scientific Publication (In Press).

B. Summary Reports

1. FREDERICK CANCER RESEARCH CENTER

The Viral Oncology Program (VOP) at the Frederick Cancer Research Center (FCRC) will conclude its third year of operations on June 25, 1975. Program direction for the VOP is derived from the Associate Director for Viral Oncology with recommendations from the FCRC Viral Oncology Advisory Group, consisting of seven NCI staff members including the FCRC Scientific Coordinator for Viral Oncology (SCVO) plus at least three outside consultants; program implementation is the responsibility of the SCVO who is located at Frederick. The major function of the FCRC Viral Oncology Advisory Group is to review all VO projects at FCRC on a continuing basis for the purpose of attaining and maintaining program excellence and to insure rapid response of the FCRC project operations to needs related to the overall NCI program objectives. The attainment of research objectives is further served through close associations between the FCRC Scientific Coordinator for Viral Oncology, and the NCI Project Monitors, the Contractor's Project Managers, the Office of Program Resources and Logistics, the Office of Biohazards and Environmental Control, and other NCI program elements.

By the end of the third year, VO/FCRC work will have been performed within several projects, which were grouped into four main areas of operation at the start of the year. These include the (1) Production Area, which contained Project 1, Virus and Viral Reagent Production, for mass producing viruses and viral components to meet program needs; (2) the Research Area, which contained Project 2, Developmental Research, to adapt or create new techniques to grow viruses that are difficult to propagate, and Project 13, Basic Research in Viral Oncology, to conduct an NCI/Contractor collaborative program of basic research in direct support of existing VO programs pertaining to the role of viruses in the etiology of human neoplasms; (3) Product Characterization and Standardization Area, designated as Project 3, to perform quality control tests on biological materials produced in Project 1; and (4) Biohazards and Environmental Control Area, to conduct a program of applied research related to the safe handling of hazardous biological materials. In addition to these work projects, Viral Oncology partially supports FCRC effort in Project 4, Environmental Control, and Project 12, Animal Breeding. Project 6 previously designated as the Advanced Systems Laboratory, which received support from Viral Oncology in past years, was redesignated as the Basic Science Program, functioning independently of the Viral Oncology Program this year. Finally, new this year, an Advanced Workshop was held at FCRC as part of the National and International Cancer Laboratory Liaison Program. Recognized national and international experts were invited to participate in a workshop-symposium, organized by VO/FCRC personnel in collaboration with the contractor, to address and recommend solutions for problems related to the production, concentration, and purification of Epstein-Barr virus and viral antigens.

Projects completely funded by Viral Oncology are the following:

1. Production Area - Project 1, Virus and Viral Reagent Production. The objectives of this project were to attain a production level of 250 L of high quality Rauscher murine leukemia virus and woolly monkey fibrosarcoma

virus per week, to concentrate and purify this, and other oncogenic or suspected oncogenic material, particularly that which would have been derived from human sources, to phase in the large-scale production of a third and fourth virus (mouse mammary tumor virus, gibbon ape virus), to produce and concentrate limited amounts of specially requested viruses on a one-time basis, to prepare special viral diagnostic and test reagents from produced viruses, and to distribute virus products as requested by the Office of Program Resources and Logistics, NCI.

2. Research Area - Project 2, Developmental Research. The objectives of this project were to continue to improve techniques applicable to scaling up cell culture production of EBV and MMTV including the testing of different strains (e.g. transforming or lytic EBV, high producing cloned cell lines) involving studies for continued optimization of parameters of growth, e.g. amino acid concentrations, ORP, pH, and dissolved O_2 and CO_2, virus induction using IdU, hormones, DMSO or cyclic AMP, and host range testing of cell cultures for infectivity and replication for improved sensitivity of assay; to develop techniques for the isolation, purification, and characterization of major (and minor) structural proteins and components; to pursue explorative studies at FCRC on cultured cells originally derived from a human liposarcoma, in collaboration with VO in-house scientists and outside expert investigators, for elucidation of problems related to verification of cell origin, methods of virus replication, and isolation and species identification of viral enzyme and structural components by serological and biochemical techniques.

Project 13, Basic Research in Viral Oncology, consists of intramural research conducted with contractor personnel on a collaborative basis as indicated below:

Subproject 13A.1 To conduct a program in support of Dr. Albert Sabin to elucidate the role of herpesvirus induced nonvirion antigens in certain forms of human cancer. Specifically: to (1) prepare nonvirion HSV antigens in guinea pig kidney and HEP2 cultures, (2) prepare virion antigens in HEP2 cultures, (3) prepare antisera in guinea pigs to the HSV nonvirion antigen(s), (4) absorb and prepare human sera for complement fixation tests, and (5) CF test large numbers of human sera from patients with a variety of cancer types.

Subproject 13A.2 Virus Disease Modification. The objective of this work was to conduct a multidisciplinary study on the detection, prevention and/or control of viral-induced and spontaneous neoplasias by (1) biochemically assessing active compounds on the basis of inhibitory effects on viral polymerase, (2) in vitro assay of test compounds for anti-viral and/or immunostimulation effects, and (3) in vivo protection and/or anti-tumor activity.

Subproject 13A.3 Isolation and Characterization of Virus-Augmented Tumor Transplantation Antigens. Objectives of this effort were (1) to purify the tumor specific transplantation antigen induced by SV40 and to study its protective capability, (2) to study the expression of virion antigens in a HSV-transformed cell system and to purify these antigens for potential use as immunogens, (3) to produce an immunogen against transplantable mammary adenocarcinomas in mice, and (4) to develop better methods for identifying and quantitating aminosugars by gas chromatography.

Subproject 13A.4 Primate Virus Section. Objective of this work was to conduct studies to develop and staff facilities for the study of the virology, biochemistry, immunology, and pathology of human and non-human primate oncogenic viruses in support of the Primate Virus Section. The laboratory is oriented to conduct and devote its efforts toward the role of human and non-human primate viruses in the induction of neoplasia, and all its studies have either direct or indirect relevance to the understanding and prevention of cancer in humans.

Subproject 13A.5 Transformation with DNA Viruses and Chemical Carcinogens. Objectives of this research were to characterize a transformed mouse cell line (H238) relating to HSV.2 gene expression to the transformed phenotype, (2) identify the HSV.2 antigens present in transformed cells and to compare these antigens with antigens synthesized by HSV.2 during the lytic infection, (3) study the immune response of the host to the HSV.2 transformed cells by analysis of HSV.2 specific antibody produced by the host following inoculation of tumor cells, (4) investigate the role of any endogenous RNA tumor viruses in these transformed cells or tumors, (5) study the susceptibility of H238 cells to superinfection by homologous virus, (6) study the infectious nature of HSV DNA and attempt to rescue HSV infectious DNA from the transformed cells, and (7) assess the transduction potential of HSV genes present in transformed cellular DNA.

Subproject 13A.6 Influence of Host Physiology on Oncogenesis and Cellular Control. The objectives of this new work will be to provide collaborative support for an on-going NCI effort directed toward studying the physiological role of endogenous xenotropic viruses and the control of their expression. The approach will be multifaceted in order to cover the effects of both host-graft and steroid-gene responses.

Subproject 13A.7 Developmental Electronmicroscopy. The objectives of this work were to (1) develop more useful methods of utilizing the electron beam for quantitative and qualitative measurements of biological specimens with the transmission (TEM) and scanning (SEM) electron microscope and to develop the appropriate techniques for preparing biological samples for these measurements, (2) study the surface features of cultivated human tumor cell lines at the ultrastructure level with the SEM and TEM using new techniques of specimen preparation, (3) initiate a research program using the SEM and TEM coupled with energy-dispersive X-ray analysis and immunological methods of specimen preparation directed towards the ultimate identification of oncogenic viruses or subviral components, (4) perform collaborative research with scientists in other areas of the FCRC to investigate the effects of certain chemical compounds on virus production, and (5) participate in a scientific exchange visit with a French electronmicroscopy laboratory.

Subproject 13A.8 Role of Endogenous Mouse Type C RNA Viruses in Naturally Occurring Disease. Objectives of this newly initiated work were to furnish facilities and personnel for (1) holding animals for studies on the spontaneous release of endogenous viruses and/or induction of leukemia, (2) chemical induction of endogenous viruses and/or leukemia, and (3) X-ray

induction of endogenous viruses and/or leukemia; to obtain clinical specimens for immunologic, virologic, and biochemical investigations, to perform gross pathology on experimental animals and to prepare specimens for histopathology upon request.

Subproject 13B National and International Cancer Laboratory Liaison. Objectives of this program were (1) to provide laboratory facilities and training for visiting foreign scientists, (2) to serve as a collaborative expert laboratory functioning within the International Agency for Research on Cancer, and (3) to plan and conduct international workshops related to current problems in cancer research.

3. Product Characterization and Standardization Area - Project 3. Objectives of this work were to provide a capability for the evaluation of all products either used or distributed within the Viral Oncology Program at FCRC and to outside investigators. Specifically to (1) develop quantitative and qualitative standards for virus, virus components or tissue culture cell lines developed and produced within the Viral Oncology Program at FCRC, (2) provide biological, biochemical, immunological, biophysical tests and essential test results necessary for the completion of program responsibilities, and (3) provide for the quality assurance and homogeneity of the virus or viral component, to determine that material shall be free of extraneous bacteriological, fungal, or mycoplasmal components by reasonable testing.

4. Biohazards and Environmental Control Area - Project 5. The objectives of this work were to conduct studies in support of the NCI Office of Biohazards and Environmental Control on (1) chemical disinfection of model oncogenic viruses, (2) development and evaluation of containment systems and monitoring procedures, (3) biological hazard assessment, and (4) personnel safe practices.

Additional projects partially supported by Viral Oncology are the following:

Project 4, Environmental Control. Objectives of this work were to develop and implement an environmental control and laboratory safety program for the FCRC complex to assure the safety of the worker, the community and the integrity of all developmental, production, and research activities, to formulate a safety policy and publish a safety and health manual for the NCI activity at FCRC, to include certification and monitoring of (1) primary containment barrier systems (safety cabinets, hoods, etc.), (2) secondary barrier systems (autoclaves, air locks, etc.) and all architectural barriers. Viral Oncology supported approximately 35 percent of this effort.

Project 12, Animal Breeding. The contractor continued to operate the Animal Farm in accordance with procedures developed and implemented previously for the breeding of rodents to meet needs of FCRC and other NCI operations as production permits. Estimated breeding requirements necessitated the operation of 22 breeding buildings before the end of the third contract year. Viral Oncology supported approximately 25 percent of this effort.

The FCRC Viral Oncology Program will be restructured next year to create the following Laboratories: (1) DNA Virus Laboratory, (2) RNA Virus Laboratory, (3) Disease Control Laboratory, and (4) Virus/Reagent Production and Developmental Research Laboratory. It will be the function of these Laboratories to conduct basic or exploratory research on programs that are or could be vital to the Cancer Program. The research in the Laboratories will include some activities previously performed under Projects 1, 2, 3 and 13, and should take full advantage of unique opportunities at the FCRC to perform investigations for which a program need exists. In addition, the contractor will provide necessary facilities and support services for viral oncology investigators whose NCI operations are located at FCRC. Also as part of the FCRC Viral Oncology Program, there will be included the effort on applied research for Biohazards and Environmental Control, previously designated as Project 5.

It should be reemphasized that the FCRC Viral Oncology effort is to be performed as a total integrated activity that will allow for a high degree of flexibility in response to overall program needs and for broad latitude in creating and pursuing fundamental research objectives both in-house and on a collaborative basis with outside acknowledged experts. As work in the given areas is performed, particular research objectives may receive changes in emphasis; objectives may be modified and/or previously established ones postponed or eliminated.

2. Office of the Associate Director for Viral Oncology

a. OFFICE OF BIOHAZARD AND ENVIRONMENTAL CONTROL

The Office of Biohazard and Environmental Control is responsible for developing and administering safety standards to prevent occupationally-acquired cancer and to protect the general environment. This office also assists contractors and where applicable, intramural and grantee programs in carrying out cancer research safety policies. Serves as a source of information, education and technical assistance on matters of cancer research safety. Collects, maintains and when necessary, assays serum of laboratory workers for possible serum conversion due to accidental exposure during laboratory manipulations. Performs research to develop effective control practices by supplementing safety standards with sufficient laboratory data and establishes risk assessment criteria. It conducts basic research pertaining to the physiological and environmental factors that alter the host's susceptibility and response to oncogenic viruses and carcinogens.

The Biohazard Research Section conducts research to elucidate the mechanisms involved in host immunocompetence and the consequences of endocrine imbalance as a normal physiological factor controlling gene expression. These factors are being investigated by in vivo and in vitro, as are the genetic factors associated with hormonal activation of protein synthesis associated with type C virus expression.

In collaboration with the Southwest Foundation for Research and Education (a collaborative contract laboratory) we are continuing to investigate the presence of type C viruses in normal baboon and human placental tissue. The significance and function of type C viruses in reproductive tissue of primates as well as rodents is being further evaluated. Conditions normally necessary for the expression of such particles are also under study. We are further exploring the nature and significance of xenotropic type C viruses.

The Environmental Control Section develops and recommends equipment, facilities, procedures and standards for the proper handling of potentially biohazardous materials and disseminates this information to the scientific community through publications, training programs, site visits and consultation activities. Procedures, equipment and facilities are evaluated to identify inherent safety difficiencies and to provide basic information for developing corrective measures. The Section operates the NCI virus containment facility and prototype containment laboratories.

The Section conducts applied research to evaluate potential hazards associated with research activities in viral and other areas of oncology and to develop adequate protective measures. This program is performed at the Frederick Cancer Research Center.

Minimum Standards for Biological Safety and Environmental Control have been issued to all contractors within the VCP. Consultation and training activities have been expanded to assist contractors and other research organizations in implementing these standards.

The Section has provided technical assistance to the NCI Cancer Research Safety Committee and to the DHEW Carcinogen Standards Committee in support

of their efforts to establish comprehensive safety standards for research involving oncogenic viruses and chemical carcinogens.

Serial No. Z01-CP-04800-03-ADVO

1. Office of the Associate Director
   Viral Oncology, Division of Cancer
   Cause and Prevention
2. Office of Biohazard and Environmental
   Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of Facilities and Procedures to Reduce Biohazardous Exposures

Previous Serial Number: Same

Principal Investigator: Mr. Manuel S. Barbeito

Other Investigators: Dr. W. Emmett Barkley

Cooperating Units: Outside NIH:
Litton Bionetics, Inc.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

The objectives of these projects are to: (1) develop an animal room containment system to prevent intercage cross-contamination and provide a safe work environment for the investigator to perform routine animal inoculations, examinations and animal care procedures, (2) identify sources and mechanisms of tissue culture contamination and develop tissue culture contamination control methods, and (3) determine the residual amount of formaldehyde in a Class II (Laminar Flow) cabinet following decontamination and its effect, if any, on tissue culture cells and on Rauscher Leukemia Virus (RLV).

Methods Employed:

The animal room containment system is a modular cubicle designed for installation within existing facilities to handle research animals including non-human primates. Containment of aerosols is achieved by using a high velocity airflow into the cubicle and recirculating the conditioned air after it has passed through high efficiency filters. Evaluation of the animal containment system was achieved by: balancing the unit for an average

airflow of either 100 or 50 ± 10 lfpm; conducting smoke particle challenge studies; performing aerosol studies using T1 coliphage with aerosol sampling equipment; determining horizontal transmission between adjacent cubicles using Newcastle Disease Virus (NDV) in 4-week-old chicks, and Sendai in 4-week-old mice, and assessing if nude (athymic) mice could be housed in the system without developing upper respiratory diseases resulting in death. T1 coliphage was developed and used as a model tissue culture system to evaluate contamination sources, levels, and handling techniques necessary within the room environment, and as a surface contaminant to cause contamination within tissue culture systems during routine manipulations. A comparison was made when working on the open bench, in a Class I cabinet, and in a laminar flow cabinet employing tissue culture techniques.

Class II (Laminar Flow) cabinets were decontaminated with formaldehyde gas and the residual concentration of gas following aeration was determined at various time intervals. The effect various concentrations of formaldehyde gas had on tissue cells and RLV were determined.

Major Findings:

A prototype animal containment cubicle test system has been designed, fabricated, and installed at the Frederick Cancer Research Center. Performance tests have been conducted to demonstrate compliance with the design criteria and specifications. The tests have shown that the animal containment system can be: balanced either at 100 or 50 ± 10 lfpm airflow rate; the pattern of particle travel as determined with smoke particles is inward without cross-contamination to adjacent cubicles; aerosols of T1 coliphage are contained within cubicles without cross-contamination to adjacent cubicles at 100 lfpm, a minimum amount of cross-contamination occured from only two lower cubicles with a reduced flow of 50 lfpm; NDV was not transmitted horizontally to control 4-week-old chicks contained in adjacent cubicles at 100 or 50 lfpm, and Sendai Virus was not transmitted horizontally to mice in cages placed on shelves in the same cubicle or to mice in cages in adjacent cubicles at 100 or 50 lfpm. Nude (athymic) mice survive at least a 65 day holding time in the containment system. The test data show that aerosols are not released from the containment system when it is balanced at either 100 or 50 lfpm. The system is therefore capable of preventing cross-contamination between adjacent cubicles and provides protection for the animal handler. Normal animal care procedures, examination and inoculations may be performed in the cubicles without causing horizontal transmission among amimals. The effect microbial room contamination has on tissue culture plates was evaluated. The minimum number of aerosolized T1 coliphage necessary to contaminate tissue cultures while working in/on (1) the Class I cabinet was 17 pfu/cf, (2) the open bench was 35 pfu/cf, and (3) the laminar flow cabinet was 248 pfu/cf. Test were conducted to determine if surface contaminants on materials used in tissue culture procedures contributed to contamination of the cell lines. When T1 coliphage was placed on the materials at concentrations from 100 to 1000 pfu/item, and routine manipulations were performed, the test showed that surface contamination was not a source of contamination

for tissue culture systems when using good techniques. When surface contamination of petri dishes was evaluated with a stock concentration of 2×10^6 pfu/ml of phage, the level of contamination of the culture media was within 3% for each operating mode. When the stock concentration was increased to 2×10^7 pfu/ml the contamination of plates in the Class I cabinets increased 25% over the open bench and 15% over the Class II cabinet. The data shows that an excessive amount of surface contamination must be present on materials to contaminate culture media. These tests demonstrate that (1) the source of contamination through the building ventilation system is a highly unlikely mode of tissue culture contamination, (2) the Class I cabinet is not a suitable working area for manipulation of tissue culture systems, (3) the open bench is a totally unacceptable work facility for use with tissue cultures, and (4) the laminar flow cabinet, when certified and operated correctly, is recommended for work with low and moderate risk oncogenic viruses. Evaluation of technician's techniques demonstrated that in the laminar flow cabinet, tissue culture plates became contaminated prior to flasks. In the Class I cabinet, the incidence of flasks contamination was greater than plates. On the open bench there is no significant difference between the rates of contamination of the tissue flasks or plates. These tests demonstrated that contamination of tissue culture systems is the result of poor operating procedures. In general, the Class II (Laminar Flow) cabinet affords the greatest product protection.

Class II (Laminar Flow) cabinets are effectively decontaminated with formaldehyde gas. The latter is generated by depolymerizing paraformaldehyde at 425F with a concentration of either 0.2 or 0.3 g/ft^3 of cabinet space and a relative humidity of above 50%. The level of formaldehyde gas, remaining at the work level following periods of aeration, was determined and the effect any residual gas may have on tissue culture cell lines and on RLV was evaluated. The data from four replicate tests shows that if the concentration of formaldehyde gas, after 24 hours aeration, is less than 0.115 µg/l (0.094 ppm) there is no apparent toxic effect on cells, or RLV. The concentration of formaldehyde gas is dissipated more rapidly in the first four hours of aeration when 0.2 g per ft^3 of space is depolymerized than when compared to 0.3 g. However, after 24 hours aeration there is no appreciable difference between the residual formaldehyde gas levels, indicating either initial concentration of paraformaldehyde may be used to decontaminate the Class II cabinet. Aeration periods of 5 hours following decontamination is not sufficient to remove residual formaldehyde gas. A small amount of formaldehyde gas (less than 0.02 µg/l) is released probably from the filter, if aeration of a cabinet is stopped prior to 48 hours post decontamination. This residual gas concentration, is not sufficient to affect tissue culture cells and RLV.

Significance to Biomedical Research and the Program of the Institute:

Results from these studies will improve the quality and safety of laboratories engaged in cancer virus research. The animal containment room project and disposal of contaminated waste will provide basic data to establish practical design and operating procedures for the safe handling of research animals and their associated waste. The data from the tissue culture contamination indicate that sources of contamination are not related to the room environment as frequently blamed, and that technique and probably latent expression of contamination in nutritive materials are most likely the sources of contamination. These tests indicate that need for good quality control over tissue culture supplies. The data determining the residual formaldehyde gas concentration following decontamination of a cabinet and its inability to affect tissue culture cells or oncogenic viruses should make it clear that residual formaldehyde gas in a hood is not a problem, providing aeration is maintained in a hood for at least 24 hours after decontamination.

Proposed Course:

A manuscript is being prepared on the data from evaluating the animal containment system.

The environmental and physical aspects of tissue culture contamination are completed and a manuscript will be prepared.

Replicated tests will be completed on the rebounding affect of formaldehyde gas following decontamination of tissue culture cells and oncogenic viruses.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04804-01-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Research Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Type C RNA-viral Expression in Mammalian Tissues

Previous Serial Number: None

Principal Investigator: Dr. George A. Saviolakis

Other Investigators: Dr. James E. Strickland
Dr. Arnold K. Fowler
Dr. Alfred Hellman

Cooperating Units: Inside NIH:
None

Outside NIH:
None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To elucidate the control mechanisms for expression of type C RNA-viral genes by steroids under physiologic conditions, to study the expression of such genes in early embryonic development and to determine the function that such genes may have in the control of cellular differentiation.

Methods Employed:

DNA complementary to the RNA of murine RNA viruses (cDNA) has been synthesized by means of the endogenous reaction of RNA-directed DNA polymerase and characterized by sedimentation velocity gradients, gel filtration

chromatography and DNA-RNA molecular hybridization. The DNA-RNA hybrid has been purified by hydroxylapatite chromatography and nuclease S_1 hydrolysis.

Major Findings:

Studies have optimized the conditions of synthesis and analysis of the cDNA. Preliminary application of these methods to the investigation of RNA obtained from spleens of Balb/c mice infected with Rauscher Leukemia Virus, has demonstrated that this cellular RNA is composed of two RNA species based on their rate of hybridization.

Significance to Biomedical Research and the Program of the Institute:

Many normal mammalian tissues contain genetic information associated with RNA tumor viruses, as indicated by the presence of proteins similar to certain components of the type C viruses and by the homology of cellular DNA (provirus) or RNA to the cDNA product of the endogenous RNA-directed DNA polymerase reaction. Moreover, the expression of this genetic information can be induced in tissues of some species by steroid hormones. The elucidation of control mechanisms for theses genes under physiologic conditions and at various stages of cellular differentiation may offer means of therapeutic intervention in states of abnormal cellular control, in which such genes may be involved.

Proposed Course:

Evidence exists that in animals of some species treated with estrogens the levels of viral (or viral like) proteins in uterine tissue increase, and that these levels vary with the estrous cycle and the gestational stage of the animal. Experiments using an estrogen radioimmunoassay will be performed to correlate tissue levels of estrogen to the degree of expression of such viral proteins. DNA-RNA hybridization will be employed to determine if estrogens modify the transcription and/or translation of the viral genes coding for such proteins. The site of action of the estrogens in the nucleus will also be explored by investigating their association with the reiterated or unique sequences of the cellular genome of the early embryo and of the mature animal. Attention will be directed to the presence of viral genes in the sequences of the cellular genome which contain estrogen-binding sites.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04805-05-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Research Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interaction of the Host with Type C Viruses

Previous Serial Number: Same

Principal Investigator: Dr. Alfred Hellman

Other Investigators: Dr. Arnold K. Fowler
Dr. James E. Strickland
Dr. Bill N. Sun
Dr. Nicola M. Kouttab

Cooperating Units: Inside NIH:
Dr. Paul T. Peebles, VLLB, NCI
Dr. A. J. Dalton, VO, NCI

Outside NIH:
Southwest Foundation for Research and Education
San Antonio, Texas
Dr. R. Gilden, Flow Laboratories, Rockville, Maryland

Man Years:

Total:	0.4
Professional:	0.4
Others:	0.0

Project Description

Objectives:

1. To determine the significance of type C viruses in pathology and physiology.

2. The evaluation of host immunocompetence as related to xenotropic viral influence and fetal development.

Methods Employed

Utilizing murine and primate model systems, studies of cellular and humoral immunocompetence of the host as well as the physiological and pathological significance of type C viruses continues. Standard cell culture, biochemical and immunological techniques are also being used.

Major Findings:

1. The influence of xenotropic viruses and their potential for mutation from host controlled messengers, that may influence tissue differentiation during embryological development and/or be involved in the acceptance of the fetus by the mother as an allograft, to an agent involved etiologically in malignancies is being investigated in the mouse and certain subhuman primates. We have clearly demonstrated that the hormonal milieu of the uterus can be modified so that type C xenotropic viruses are expressed in vivo. Such viruses have been isolated by us from estrogen stimulated NIH-Swiss mice and are presently being characterized as to their biological functions. Numerous genetic studies have suggested the involvement of host genes in expression of type C viruses. The mechanisms by which these genes are activated and repressed is poorly understood. Our evidence favors the interpretation that estrogen induces in the uterus de novo synthesis of antigens which are integral components of type C RNA viruses. The AKR/N mouse, a high leukemia strain, readily expressing type C viral information is not controllable by estrogen modulation. In contrast, the C57L mouse, a low leukemia strain, demonstrates highly suppressed type C viral expression which is not apparently influenced by estrogen manipulation. The NIH-Swiss and Balb/cAnN mouse, intermediate in their incidence of leukemia, can be induced by estrogen to express xenotropic type C viruses. This type of information suggests to us that estrogenic activation of type C viruses is under genetic control, amenable to physiological modification. It is interesting, but perhaps coincidental, that both in man, subhuman primates, rabbits and mice, type C viral particles are observed in tissue under appreciable hormonal influence. It is recognized that the uterus serves more than a supportive function in embryonic development. In the absence of intact uterine environment the embryo does not develop beyond the blastocyst stage. In order to progress beyond this stage a factor capable of transmitting information from the mother to the embryo is thought to be essential. Such a messenger system would have to fulfill certain criteria such as: species specificity; induction of genetic and/or metabolic activity; readily transmissible from mother to fetus; and inducible at the proper time and under specific physiological controls; type C particles described by us in primates and mice, meet all the criteria necessary to serve as cell to cell communicators and gene amplifiers.

2. In the subhuman primate and man it has been determined that type C viruses are present. In extensive studies of the baboon, endogenous type C viruses have been observed and isolated from placentas, embryonic and adult tissues. These isolates in the most part have been characterized as xenotropic viruses. However, in the case of the M-7 virus, a RD-CCC-primate-cat virus, growth has

been demonstrated in cells from the species of origin (baboon). All isolates to date are immunologically and biophysically related and in turn have similarities to the CCC-RD-114 viruses. They replicate in a variety of cells in vitro including those of human, ape, Old World monkey and dog. Even though inoculation of the M-7 virus into several subhuman primates, hamsters and nude mice did not apparently infect these animals, inoculation of the M-7 virus into beagle dogs has successfully infected this species. Thus far the M-7 virus has been reisolated from tissue cultures of such dogs, inoculated in utero and the dogs have demonstrated an immunological response to M-7. It should be pointed out that this is the first demonstrable horizontal infection of a mammal by a xenotropic primate-cat virus. Baboon studies clearly suggest that these viruses are primarily vertically transmitted, since they have been demonstrated to occur in baboon follicular oocytes and tubal ova. Autogenous humoral and cellular immunity in the baboon, similar to that seen in the mouse, directed against the xenotropic type C viruses, has been observed.

Significance to Biomedical Research and the Program of the Institute:

The elucidation of the events leading to xenotropic type C marker expression in vivo as well as its implication in immunocompetence and normal physiology provide a rational means for determining the significance of such particles in oncogenesis. An understanding of inducing or co-inductive factors associated with malignancy will provide for a more meaningful means of control and prevention.

Proposed Course:

1. Clearly these in vivo systems are amenable to and will continue to be investigated in order to determine the function of these viruses and their possible evolution from physiologically controlled messengers to etiological involvement in disease.

2. The development of in vitro assay systems by studying early inductive changes during viral expression and to determine the genetic and immunologic factors associated with such induction and its modification will continue.

Honors and Awards:

None

Publications:

Dalton, A. J., Hellman, A., Kalter, S. S. and Helmke, R. J.: An Ultrastructural Comparison of Placental Virus with Several Type C Oncogenic Viruses. J. Nat. Cancer Inst. 52: 1379-1381, 1974.

Fowler, A. K., Kouttab, N. M., Kind, P. D., Strickland, J. E. and Hellman, A.: Oncornaviral Protein Modulation in Mouse Uterine Tissue by Estrogen. Proc. Soc. Exp. Biol. Med. 148: 14-18, 1975.

Hellman, A., Peebles, P. T., Strickland, J. E., Fowler, A. K., Kalter, S. S., Oroszlan, S. and Gilden, R. V.: Baboon Virus Isolate M-7 with Properties Similar to Feline Virus RD-114. J. Virol. 14: 133-138, 1974.

Kalter, S. S., Panigel, M., Kraemer, D. C., Heberling, R. L., Helmke, R. J., Smith, G. C. and Hellman, A.: C-type Particles in Baboon (Papio cynocephalus) Preimplantation Embryos. J. Nat. Cancer Inst. 52: 1927-1928, 1974.

Strickland, J. E., Kind, P. D., Fowler, A. K. and Hellman, A.: Comparison of Viral Marker Proteins in Murine Leukemia Virus and Mouse Uterus. J. Nat. Cancer Inst. 52: 1161-1165, 1974.

Hellman, A., Fowler, A. K., Strickland, J. E. and Kouttab, N. M.: Type-C Virus Modulation by Estrogen: Its Possible Biological Function. In Rainer, H. (ed.): Molecular Basis of Malignancy. (In press).

Kalter, S. S., Heberling, R. L., Hellman, A., Todaro, G. J. and Panigel, M.: Viruses in the Transmission of Cancer: C-type Particles in the Baboon Plancenta. Proc. Roy. Soc. Med. (London) (In press).

Kalter, S. S., Heberling, R. L., Helmke, R. J., Panigel, M., Smith, G. C., Kraemer, D. C., Hellman, A., Fowler, A. K. and Strickland, J. E.: A Comparative Study on the Presences of C-type Viral Particles in Placentas from Primates and Other Animals. Comparative Leukemia Res. (In press).

Kalter, S. S., Heberling, R. L., Smith, G. C., Panigel, M., Kraemer, D. C., Helmke, R. J. and Hellman, A.: Vertical Transmission of C-type Viruses: Their Presence in Baboon Follicular Oocytes and Tubal Ova. J. Nat. Cancer Inst. (In press).

Heberling, R. L., Kalter, S. S., Barker, S. T., McCullough, B., Smith, G. C., and Hellman, A.: Infection of Beagles with Endogenous Baboon C-type Virus. Science. (In press).

Serial No. Z01-CP-04806-03-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Evaluation and Development of Biological Safety and Environmental Control Equipment

Previous Serial Number: Same

Principal Investigator: Dr. W. Emmett Barkley

Other Investigators: None

Cooperating Units: Outside NIH:

The Dow Chemical Company
Frederick Cancer Research Center

Man Years:

Total:	0.25
Professional:	0.25
Other:	0.00

Project Description

Objectives:

The objectives of the biological safety and environmental control equipment project are: (1) to develop primary barrier systems for the containment of oncogenic viruses, (2) to establish in collaboration with the NIH Procurement Branch and the Environmental Services Branch a Qualified Products List for safety equipment to assure compliance with safety performance standards, (3) to evaluate equipment performance testing methods and develop standardized testing procedures, and (4) to assess hazards associated with the use of research equipment and procedures.

Methods Employed:

Standard engineering methods for the measurement of airflow, air velocity and filter penetration are employed. Physical and biological tracer assay systems are used to evaluate equipment performance. Environmental sampling procedures are routinely employed.

Major Findings:

Certification studies were conducted for five NIH contracts for the procurement of Class II biological safety cabinets. These studies demonstrated design and fabrication problem areas that could adversely affect the safety performance of these containment devices. The most serious problem areas were associated with the fabrication of the gas tight recirculation ducting systems and the selection of fan and motor assemblies. This testing program has demonstrated the necessity of performing certification tests on preproduction containment equipment in order to assure that equipment purchased to contain biohazards can achieve the specified level of safety performance.

Studies were performed to determine the product protection capability of the NCI designed Class II Type II biological safety cabinet. In one study, room cross flow currents of 50 and 100 lfpm were established to simulate activity that might be anticipated adjacent to the safety cabinet. Results indicated that these adverse room conditions would not compromise product safety when the cabinet was operated with the cabinet view window in its normal position. Minimal work area contamination was observed when the access opening was greater than 15 inches. Fewer than 300 test spores were recovered in the work area of the cabinet under the most adverse operating conditions during a test challenge of 1×10^6 spores. These results indicate that the Class II Type II biological safety cabinet can prevent airborne contamination of research materials under anticipated adverse room conditions.

A test to measure the lateral spread of contaminants released within the enclosure of Class II biological safety cabinets was developed. This test will be adopted by the National Sanitation Foundation as one of the Quality Assurance Specifications for Class II safety cabinets.

Significance to Biomedical Research and the Program of the Institute:

This project will improve the performance capabilities of safety equipment used for the containment of potentially hazardous biological materials. The establishment of a Qualified Products List for safety equipment and the performance testing of preproduction equipment will insure that safety equipment procured for biohazard containment will meet necessary safety standards. Assessing the inherent hazards of research equipment and procedures will allow practical selection of containment methods to assure personnel safety.

Proposed Course:

The testing methods developed in this project will be used to evaluate the safety performance of preproduction and prototype containment equipment. Additional testing methods will be developed to monitor the operating efficiency of this equipment under laboratory use conditions. These methods will also be used to determine the aerosol hazard potential of various

research procedures and activities. These studies will be conducted in the future by contract personnel at the Frederick Cancer Research Center.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04807-05-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Research Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunocompetence and Susceptibility of Animal Systems to Oncogenesis

Previous Serial Number: Same

Principal Investigator: Dr. Arnold K. Fowler

Other Investigators: Dr. Alfred Hellman
Dr. Nicola M. Kouttab
Dr. James E. Strickland
Dr. Bill N. Sun

Cooperating Units: Inside NIH:
None

Outside NIH:
Southwest Foundation for Research and Education, San Antonio, Texas

Man Years:

Total:	2.4
Professional:	1.4
Other:	1.0

Project Description

Objectives:

To define and characterize physiological and genetical mechanisms contributing to host immunological regulation and to elucidate their role in oncogenic expression. To examine the significance of detecting oncornaviral information in reproductive tissues and the relevance of hormonal and/or gestational events in modifying its expression.

Methods Employed:

The in vivo and in vitro activation of virus or oncornaviral protein expression by a variety of physiological stimuli is being characterized using conventional methods: group specific proteins of the type C RNA tumor viruses (gs-1, gs-3) by radioimmunoassay, complement fixation, immunofluorescence, and gel precipitation techniques; RNA-directed DNA polymerase by biochemical, biophysical, and serological methods; virus synthesis by electron microscopy and in vitro cocultivation assays. These criteria of oncogenic detection are being studied concomitantly with various parameters of host immunocompetence. These include the in vivo maternal/fetal interactions and delayed hypersensitivity, as well as in vitro two-way and one-way mixed lymphocyte reactions and the in vitro response of lymphocytes to specific and nonspecific antigenic stimulation.

Major Findings:

Treatment of ovariectomized NIH-Swiss mice with estrogens elevates the level of murine leukemia virus group specific proteins and the activity of an RNA-directed DNA polymerase in the uterus. The extent that these oncornaviral protein levels are raised is dependent on the relative biological potency of the estrogen and on the time interval following treatment. Estradiol-17β, the most potent of the metabolites examined, was most effective in elevating gs protein; whereas, estradiol-17α, the least potent, was least effective in raising gs protein level. The effectiveness of estrone and estriol were intermediate and reflected their relative biological potencies. Increases in the levels of both viral proteins were evident within 12 hrs of estradiol-17β treatment and were highest at 48 hrs. Subsequently, viral protein levels declined to pretreatment levels. Evidence recently obtained using several tissue cocultivation systems indicates that the activated virus is of the endogenous xenotropic type. Electron microscopy and immunofluorescence of uterine tissue localize the virus particles within the secretory uterine epithelium. Mature particles are frequently seen in the intercellular spaces of the endometrium, as well as budding from the epithelial cells near the basal lamina. Occasionally, particles are also observed in adjacent stromal cells but, to this date, no virus particles have been seen in the lumen of the uterus.

Most recently, we have characterized a pregnancy associated variation in viral information expression in the NIH Swiss uterus. Following coitus, the level of uterine gs protein increases approximately two-fold over precoitus. The level then remains essentially unchanged until the terminal week of gestation when it is observed to increase markedly, culminating in a ten-fold increase near parturition. In coded specimens, the abundance of virus particles, observed by electron microscopy, also appears subjectively higher in the gravid uterus.

Significance to Biomedical Research and the Program of the Institute:

The essential role of host immunity, as dictated by genetic and environmental control mechanisms, in the etiology of some, if not all cancers, has become increasingly apparent during recent years. The concept that degradation of natural immunity processes under certain environmental and/or physiological stresses precedes or is concomitant with infection by oncogenic viral agents is not new, although it remains poorly defined. The frequent implication of host physiology in the development and pathogenesis of cancer, as well as their involvement in host immunocompetence, points to the need of further research directed toward elucidating the biochemical mechanisms and the degree of its participation in these phenomena as a prerequisite to the development of means to control human cancers.

Proposed Course:

The development of an in vivo system permitting the "at will" control of virogene marker expression by the manipulation of the host's endocrine milieu now provides a model to comprehensively examine the relevance of virus information in tumorogenesis. Efforts will be concentrated on physiological and immunological mechanisms as they interact with host genetics and immunocompetence in the induction and propagation of the malignant state. Furthermore, attempts will be directed toward the characterization of the underlying biochemical mechanisms of these interrelationships.

Honors and Awards:

None

Publications:

Strickland, J. E., Kind, P. D., Fowler, A. K. and Hellman, A.: Comparison of Viral Marker Proteins in Murine Leukemia Virus and Mouse Uterus. J. Nat. Cancer Inst. 52: 1161-1165, 1974.

Hellman, A., Peebles, P. T., Strickland, J. E., Fowler, A. K., Kalter, S. S., Oroszlan, S. and Gilden, R. V.: Baboon Virus Isolate M-7 with Properties Similar to Feline Virus RD-114. J. Virol. 14: 133-138, 1974.

Fowler, A. K., Kouttab, N. M., Kind, P. D., Strickland, J. E. and Hellman, A.: Oncornaviral Protein Modulation in Mouse Uterine Tissue by Estrogen. Proc. Soc. Exp. Biol. Med. 148: 14-18, 1975.

Hellman, A., Fowler, A. K., Strickland, J. E. and Kouttab, N. M.: Type-C Virus Modulation by Estrogen: Its Possible Biological Function. In Rainer, H. (ed.): Molecular Basis of Malignancy. (In press).

Kalter, S. S., Heberling, R. L., Helmke, R. J., Panigel, M., Smith, G. C., Kraemer, D. C., Hellman, A., Fowler, A. K. and Strickland, J. E.: A Comparative Study on the Presences of C-type Viral Particles in Placentas from Primates and Other Animals. Comparative Leukemia Res. (In press).

Serial No. Z01-CP-04808-02-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Research Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Interactions of Friend Virus with Immunocompetent Cells. Immunologic and Hormonal Mechanisms of Allograft Acceptance.

Previous Serial Number: Same

Principal Investigator: Dr. Nicola M. Kouttab

Other Investigators: Dr. Arnold K. Fowler
Dr. Alfred Hellman
Dr. James E. Strickland

Cooperating Units: Inside NIH:
Dr. Richard Asofsky
Laboratory of Microbial Immunity
National Institute of Allergy and Infectious Diseases

Outside NIH:
None

Man Years:

Total:	1.5
Professional:	1.2
Others:	0.3

Project Description

Objectives:

To identify the target cells for Friend virus infection and the consequence of virus-cell interaction.

To investigate the contribution of immunological and hormonal mechanisms in allograft acceptance and to examine the role of oncornaviral information in this event.

Methods Employed:

Histological techniques will be used to investigate the consequence of cell-virus interaction. These will be supplemented with techniques in cellular immunity and humoral immunity. Immunofluorescence techniques will be used to define more clearly the target cell for Friend virus.

In vivo and in vitro techniques are used to examine the mechanism for allograft acceptance. Hormonal and immunological stresses are investigated to determine their involvement in immunological inadequacy as measured by host vs graft (maternal-fetal) reactions. Other techniques include lymphocyte stimulation by mitogens and allogeneic cells in the presence and absence of physiological immunosuppressants.

Major Findings:

Immunosuppression of mice with anti-μ from birth conferred resistance to Friend virus. No leukemia was produced in these mice after Friend virus injection even after immunosuppression was stopped. It appears that at least one of target cells for Friend virus is a cell bearing μ-receptor on its surface, and may be an early stage in the antibody producing lineage.

A substance was found in uterine and placental extracts that inhibits lymphocyte stimulation by mitogens and by allogeneic cells. This substance, although present in normal uteri, increases significantly during gestation. The interaction of this substance with lymphocytes is reversible and non-cytotoxic. Due to its increase during gestation, this substance may participate in the mechanisms preventing fetal rejection.

Significance to Biomedical Research and the Program of the Institute:

Defining interactions of cells with type C RNA viruses will provide information on the nature and critical properties of "target cells" and perhaps provide an insight into the mechanisms by which viruses are capable of infecting particular cells. Furthermore, it will also provide a means to assess host immunological surveillance mechanisms modifying viral expression which participate in normal and abnormal cellular growth and differentiation.

Proposed Course:

In vitro methods will be developed to study the presence of mediators that confer protection to the fetus. Attempts will then be made to identify these mediators, and their mechanisms of action.

In vivo and in vitro techniques will be developed to provide a clear perspective of the events that lead to cell-virus interaction, the consequences of such interactions, and how these interactions can be controlled.

Honors and Awards:

None

Publications:

Fowler, A. K., Kouttab, N. M., Kind, P. D., Strickland, J. E. and Hellman, A.: Oncornaviral Protein Modulation in Mouse Uterine Tissue by Estrogen. Proc. Soc. Exp. Biol. Med. 148: 14-18, 1975.

Manning, D., Kouttab, N. M. and Jutila, J. W.: The Effect of Anti-μ on Friend Virus Infection. J. Immunol. 112: 1198-1703, 1974.

Hellman, A., Fowler, A. K., Strickland, J. E. and Kouttab, N. M.: Type-C Virus Modulation by Estrogens: Its Possible Biological Function. (In press).

Serial No. Z01-CP-04810-03-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Research Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on RNA-Directed DNA Polymerases of Mammalian Tissues

Previous Serial Number: Same

Principal Investigator: Dr. James E. Strickland

Other Investigators: Dr. Arnold K. Fowler
Dr. Alfred Hellman
Dr. Nicola Kouttab
Dr. Bill N. Sun

Cooperating Units: Inside NIH:
None

Outside NIH:
None

Man Years:

Total:	2.7
Professional:	1.0
Other:	1.7

Project Description

Objectives:

To characterize the mechanism controlling viral DNA polymerase activity with particular regard to those factors involved in the utilization of natural RNA templates as opposed to synthetic templates. To determine the response of oncornaviral gene products in mammalian reproductive tissues to normal physiological stimuli, to identify the role of viruses and viral proteins in normal reproduction and differentiation, and to elucidate the control mechanism of viral gene expression in mammalian tissues.

Methods Employed:

We have used RNA-directed DNA polymerase as our marker viral protein in conjunction with others of our group using radioimmunoassay, fluorescent antibody, and tissue culture assay techniques to detect virus and viral proteins. Various strains of mice have been used to study variations in viral protein expression in similar physiological states among animals with a range of susceptibility to spontaneous viral expression or leukemia-like diseases. Polymerases from a number of viruses, both xenotropic and ecotropic, have been compared with respect to molecular weight and immunological similarity using enzyme inhibition tests with antisera prepared against purified polymerases.

Major Findings:

Subcellular fractionation studies have shown that viral type DNA polymerase, which is expressed in the uteri of ovariectomized NIH mice in response to estrogen treatment, is primarily associated with the microsomal fraction, with lesser amounts in mitochondria. Group-specific antigen, detected by a p30 radioimmunoassay, is also predominantly in microsomes and mitochondria. Sucrose density gradient centrifugation of uterine preparations showed the polymerase activity banding in a single peak of density 1.13, while in simultaneous gradients with RLV the activity was found at 1.17. In early gestation (<6 days) when we detect high levels of viral proteins in the uterus and when maturing and budding virus particles are readily seen by electron microscopy, polymerase activity appears at a density of about 1.16 in addition to the 1.13 peak. Both p30 and viral polymerase vary in a systematic way during gestation. On a specific activity basis, these markers decrease to a low value at 9-12 days. When total content per uterus is calculated, however, there is a steep rise in gs protein level late in the gestation period. As determined by immunofluorescence and EM, viral proteins and particles appear to be localized in the secretory epithelium of the uterine endometrium.

A study of old (2.0-2.5 years) mice showed that these animals had lost the ability to respond to estrogen by increased uterine viral proteins, both p30 and polymerase, although general uterotropic response was observed. Repeated injections of estrogen over a two-week period failed to elevate oncornaviral protein levels. The baseline, untreated levels of viral proteins, however, were higher in old than in young animals.

Comparisons of DNA polymerases from ecotropic and xenotropic mouse viruses have been made on the basis of comparative inhibition by antisera made against purified polymerases from RLV and NZB virus. Partially purified polymerases from NZB virus, Balb: virus 2, AT-124 (NIH Swiss), and RLV were examined. These enzymes appeared indistinguishable from one another on the basis of inhibition by either antisera. Such a result is not unexpected if xenotropic viruses are derived from ecotropic ones by recombination or some other mechanism.

Significance to Biomedical Research and the Program of the Institute:

Many, if not all, normal mammalian cells contain structural genes for C-type RNA virus proteins. The finding of viral proteins in the uterus during gestation may suggest a role for these viruses in differentiation or other normal physiological processes. Oncogenic viruses may be variants of agents with normal developmental function. Elucidation of the mechanism whereby tissues can express or repress this information in response to physiological stimuli offers hope of manipulation of expression of viral genes for therapeutic purposes.

Proposed Course:

We plan to initiate studies to determine the mechanism by which crude RLV polymerase can use viral RNA as template but purified enzyme cannot use purified 70S RNA.

In vivo labeling experiments will be done to determine whether viral proteins are synthesized as high molecular weight precursors in the estrogen-stimulated uterus, as seems to occur in some in vitro systems.

We will collaborate with Dr. Kouttab of our group to characterize an inhibitor of lymphocyte transformation in mouse uterus during gestation and to investigate its possible relationship to viruses.

Honors and Awards:

None

Publications:

Fowler, A. K., Kouttab, N. M., Kind, P. D., Strickland, J. E. and Hellman, A.: Oncornaviral Protein Modulation in Mouse Uterine Tissue by Estrogen. Proc. Soc. Exp. Biol. Med. 148: 14-18, 1975.

Hellman, A., Peebles, P. T., Strickland, J. E., Fowler, A. K., Kalter, S. S., Oroszlan, S. and Gilden, R. V.: Baboon Virus Isolate M-7 with Properties Similar to Feline Virus RD-114. J. Virol. 14: 133-138, 1974.

Strickland, J. E., Kind, P. D., Fowler, A. K. and Hellman, A.: Comparison of Viral Marker Proteins in Murine Leukemia Virus and Mouse Uterus. J. Nat. Cancer Inst. 52: 1161-1165, 1974.

Hellman, A., Fowler, A. K., Strickland, J. E. and Kouttab, N. M.: Type-C Virus Modulation by Estrogen: Its Possible Biological Function. In Rainer, H. (ed.): Molecular Basis of Malignancy. (In press).

Serial No. Z01-CP-04813-01-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Research Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and Characterization of Endocrine Induced Xenotropic Virus and Gene Control of Hemoglobin Synthesis

Previous Serial Number: None

Principal Investigator: Dr. Bill N. C. Sun

Other Investigators: Dr. Arnold K. Fowler
Dr. James E. Strickland
Dr. Nicola M. Kouttab
Dr. Alfred Hellman

Cooperating Units: Inside NIH:
None

Outside NIH:
None

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

The primary objective is the quantitation of the expression of infectious xenotropic type C RNA virus in uterine tissue of mice, and subsequently correlating of this with the estrogen activation of a group-specific viral antigen (gs) and an RNA-directed DNA polymerase (RDDP). Whether the estrogen can activate the whole viral genome or simply some of its genes, such as viral antigen and RDDP, can be answered by the determination of their relationship in estrogen-treated mice.

To study the control mechanisms of hemoglobin induction using the interspecific hybrid between human and mouse erythroleukemia cells and to determine the effect of Friend leukemia virus on these mechanisms.

Methods Employed:

Reproductive tissues of mice are cocultivated with S+L- cells with the resulting supernatant used to infect a sparse monolayer of human amnion cells. The detection and quantitation of infections xenotropic virus is then based on foci development in the human amnion cells.

Human chromosomes are preferentially segregated in the progeny of cell hybrids formed between human and rodent cells. In an appropriate selective medium it is possible to retain a specific human chromosome carrying the wild-type allele that compensates the mutant allele in the rodent genome. Henceforth, a series of auxotrophic mutants will be induced and isolated in erythroleukemia cell with preferential emphasis on those markers which are linked with human hemoglobin genes such as chromosome 2 and b group chromosome.

Major Findings:

The sensitivity of the cocultivation method permits the detection of xenotropic virus from mice which are either estrogen-treated or ovariectomized and estrogen-treated. The pseudotypes produced from S+L- cells are cloned and replicated. Supernatant harvested from these S+L- cells shows 10-fold increase in RDDP activity indicating that NIH pseudotype is released continuously.

Drug resistant mutants lacking of hypoxanthine guanine phosphoribosyl transferase have been consistantly isolated from the erythroleukemia cell line by means of a combination of treatment 5-bromodeozyuridine and black light. This method has been successfully applied to Chinese hamster cells to isolate galactose-negative mutants.

Significance to Biomedical Research and the Program of the Institute:

Random-bred NIH Swiss mice were believed "virus free" though gs antigen appeared to be expressed in high titers in normal postnatal hematopoietic tissues as well as in embryos at 14 and 18 days. The inability of several laboratories to recover infectious virus from NIH Swiss mice, both in vitro and in vivo, has led Rowe to postulate that NIH Swiss mouse harbors a defective endogenous virus. Levy succeeded in 1973 to isolate the first xenotropic virus from NIH Swiss mice, after cocultivating the mouse organs on human cells, and then infecting transformed normal rat kidney cells with the supernatant. Xenotropic viruses were coined by him as one of the two classes of endogenous virus which can be grown in cells foreign to the host, while the ecotropic viruses can multiply in cells of the host species.

Our independent finding using both the human amnion cell and its sarcoma-transformed strain demonstrating the presence of xenotropic virus in NIH Swiss strengthens Levy's observation and provides new evidence supporting the hypothesis of inherited oncogenic information. The endogenous xenotropic virus in normal cells can be regarded as a potential cancer-inducing agent which is susceptible to being triggered by the physiological environment.

The somatic cell hybridization between mouse erythroleukemia cell and human cell not only provides an unambiguous way of mapping human hemoglobin genes on human chromosomes but also makes the study of the mechanism of human hemoglobin induction and differentiation possible and feasible in vitro. The elucidation of the mechanism involved may determine whether Friend virus participates in the cell's responsiveness to DMSO induction of hemoglobin synthesis.

Proposed Course:

A new and more sensitive cell line, showing contact-inhibition during confluency, will be employed so that quantitative studies on xenotropic virus titers from reproductive tissues of mice will be more readily isolated, quantitated and characterized.

The adaptation of the erythroleukemia cells from the conventional in vitro suspension culture system to suitable plating and cloning systems will be made as a prerequisite for these studies. With the availability of this mass culture technique it then becomes possible to induce and select auxotropic mutants.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04816-01-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of the Dispersion and Dilution of Air Contaminants in a Ventilated Space.

Previous Serial Number: None

Principal Investigator: Mr. David L. West

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

Explicit objectives of this research project are (1) to characterize the dispersion and dilution of gaseous and small-particle aerosol contaminants within a confined, ventilated space that simulates a biomedical research laboratory, or other work space, and (2) to detect and describe any deviations from the theoretical phenomena of perfect mixing of contaminant and air.

Methods Employed:

For each experimental parameter or combination of parameters, a tracer gas will be released into the test space and concurrent air samples will be taken at numerous sampling points. The collected data will be statistically analyzed to (1) determine the spatial distribution of the tracer gas concentration, (2) determine the degree of mixing at the respective sampling points, (3) test for homogeneity of tracer gas concentration at the respective sampling points.

Major Findings:

An experimental test space has been designed and is being procured. This experimental system includes a control volume (simulated laboratory) and a ventilation system that can be accurately controlled to provide the range of ventilation conditions necessary to accommodate the experimental parameters.

A tracer gas sampling and analyzing system has been designed and procured. The system is capable of (1) sampling tracer gas from one of 112 sampling points in the control volume and (2) providing either continuous or momentary measurement and recording of gas concentration at a selected point(s).

The statistical design for the collection and manipulation of experimental data has been developed in accordance with the methods outlined above.

Significance to Biomedical Research and Program of the Institute:

The dilution of an air contaminant is an important consideration in determining exposure levels and assessing health hazards of exposure to a toxic or infectious agent. Currently, exposure levels to airborne agents is calculated (1) on the assumption of perfect mixing, or (2) with arbitrarily selected mixing factors; the results of this project will assist the health science researcher and the epidemiologist in accurately evaluating personnel exposure to airborne agents.

The results of this research will (1) allow more accurate estimation of personnel exposure to accidentally released or aerosolized contaminants that may be injurious to the health of laboratory workers, (2) provide a basis for optimizing laboratory ventilation design, and consequently (3) promote the validity of diagnostic tests and the integrity of experimental studies by contributing toward the maintenance of contaminant-free work environments.

Proposed Course:

The experimental apparatus and methodology will be employed and perfected; experimental protocols will be developed for future studies of contaminant dispersion in laboratories and work spaces with diverse conditions of ventilation, contaminant release, local exhaust and containment equipment, and occupancy and personnel activity.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04819-03-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Safety and Environmental Control Survey

Previous Serial Number: Same

Principal Investigator: Dr. Garrett V. Keefer

Other Investigators: Dr. Alfred Hellman
Mr. Manuel Barbeito

Cooperating Units: Outside NIH:
The Dow Chemical Company

Man Years:

Total:	0.4
Professional:	0.4
Other:	0.0

Project Description

Objectives:

The Office of Biohazard and Environmental Control (OB&EC) has the responsibility for surveying facilities, equipment and laboratory procedures at the various collaborating laboratories within the Virus Cancer Program with the view toward recognition and reducing potential biohazards and enhancing the research environment.

Methods Employed:

The format for conducting the survey has not changed appreciably from last year. The OB&EC has assembled a group of staff personnel and consultants with individual expertise in microbiology, biohazard safety, and engineering to perform these surveys. The survey team is comprised of two or 3 individuals and is headed by a member of the OB&EC. The project officer usually accompanies the survey team to provide the link to the program and is available to answer any questions concerning funding that might arise. The team meets with the principal investigator, his staff, and facility engineer with access to current building drawings, and a member of the facilities safety group. The

Principal Investigator on the contract describes his project and identifies various laboratory functions including the support groups. The presentation usually includes: (1) the quantities and types of oncogenic viruses under study, (2) the personnel and operational practices used by laboratory personnel, (3) the availability of biohazard equipment (Class I and II cabinets, autoclaves) and (4) the degree of compliance with the "Minimum Standards of Biological Safety and Environmental Control for Contractors in the SVCP." Suggestions by the contractor personnel as to how these Standards could be improved are also noted. The OB&EC representative emphasizes the service aspect of the survey and describes other activities performed by the OB&EC in support of VCP contractors. After the meeting, the team tours the facility observing normal laboratory practices and operations. At the completion of the survey the visitors again meet with the principal investigator to identify major deficiencies and provide recommendations for their removal. Each member of the survey team submits a trip report including his observations. These are discussed and the salient points are compiled into a letter addressed to the principal investigator. Eight contractors were surveyed in the past year. Five of the surveys were initiated by OB&EC, two were performed at the request of the contractor and one at the request of Dr. John Moloney, Associate Director, VO.

Major Findings:

Results of the survey over successive years has demonstrated an increased awareness of the need for biological safety and environmental control procedures by the professional and technical personnel with a background in microbiology. In addition, laboratory personnel have a better concept of the type of containment devices needed to protect both personnel and the product. On the other hand, greater emphasis must be placed on increasing the awareness of the biochemist and his technical staff to the potential biological hazards encountered in the oncogenic virus laboratory. The survey team contributes to this indoctrination by urging that laboratory personnel attend a course presented by the University of Minnesota under contract to OB&EC. Members of the survey team also describe slide cassette-tape training packets concerning various safety topics, e.g. the proper use of the laminar flow biological safety cabinet. Another contribution of the survey is to identify areas where more information is needed in order to improve the laboratory environment. The surveys performed the past year again demonstrated a need for information concerning (1) the chemical inactivation of oncogenic viruses and their nucleic acids, (2) the testing and certification of containment equipment, (3) the assessment of risk associated with oncogenic viruses or chemical carcinogens and their manipulative processes, and (4) the inactivation and disposal of chemical carcinogens.

Significance to Biomedical Research and Program of the Institute:

The significance to biomedical research or to any research effort is that this program provides safety information and guidance which can be used as the basis for protecting the researcher and his environment.

Proposed Course:

Eight to ten contractor laboratories are scheduled for survey in the coming year. Priority will be given to production facilities and laboratories studying materials of moderate risk.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04820-02-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of a Carcinogenic Chemical Safety Program for the VCP

Previous Serial Number: Same

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. W. Emmett Barkley

Cooperating Units: Inside NIH:

Carcinogenesis Branch, DCCP, NCI

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

To develop a carcinogenic chemical safety program to complement the ongoing biologic safety program and thus provide environmental control of both chemical and viral oncogenic agents in the operations of the Virus Cancer Program.

Methods Employed:

As described in the preceding project report (July 1, 1973 through June 30, 1974), preliminary guidelines for the safe handling of chemical carcinogens were developed which were modeled after the Minimum Standards of Biological Safety for Contractors of the VCP, which had been prepared by OB&EC (July, 1972). These guidelines were further developed and issued as Minimum Safety Guidelines for Research Involving Chemical Carcinogens by the NCI Chemical Research Safety Committee in draft form on July 25, 1973. The draft form was submitted to a number of reviewers and their comments were incorporated into a greatly improved version dated January 28, 1974.

Major Findings:

The NCI Safety Guidelines for Research Involving Chemical Carcinogens served as a basis for the development of Safety Standards governing the use of chemical carcinogens in all the laboratories of DHEW, by a Subcommittee of the DHEW Toxicology Coordinating Committee. This Subcommittee on Carcinogen Standards, whose membership included two staff members of the OB&EC, prepared in draft form DHEW Safety Standards for Laboratory Operations Involving Chemical Carcinogens in April 1974. A revised document, which incorporated the suggestions and comments of additional reviewers was issued on January 8, 1975. This was submitted to a number of concerned agencies of DHEW, for their comments. On their unanimous concurrence the final document was approved by the Subcommittee on January 23, 1975, for transmittal through the parent Toxicology Committee to the Assistant Secretary for Health, DHEW, who would then in turn transmit it to the Assistant Secretary for Administration and Management, DHEW, for implementation.

An early version of the NCI Safety Guidelines for chemical carcinogens had served as the basis for the laboratory portions of the recent OSHA regulations for work with certain chemical carcinogens (Federal Register 39, 3755, 1974). At this time, these laboratory sections have been remanded by the Court back to the Department of Labor (DOL) because of procedural errors. It is anticipated that the DHEW Safety Standards will be transmitted, perhaps through NIOSH, to DOL for their use for preparing new laboratory sections of the OSHA carcinogen standards. Because of these events, the new laboratory sections of the OSHA standards will probably be much less divergent from the DHEW Safety Standards which NCI will follow than the previously published laboratory sections.

Significance to Biomedical Research and the Program of the Institute:

Safe handling of hazardous materials is of obvious importance to all research programs. The environmental control program required to accomplish this will not only provide a safeguard for laboratory workers and the general community but will also help eliminate a source of error in experimental systems (e.g., animal studies) subject to cross-contamination.

The safety procedures already developed for the VCP will become important elements of the total safety program of the NCI.

Proposed Course:

In order to meet the safety needs of other Divisions within NCI in addition to Viral Oncology and Chemical Carcinogenesis of DCCP, an Office of Research Safety in the Office of the Director, NCI is being established. This office will be staffed initially by two scientists who will be transferred from OB&EC.

Honors and Awards:

None

Publications:

None

SUMMARY REPORT

b. OFFICE OF THE COORDINATOR FOR ULTRASTRUCTURAL STUDIES
July 1, 1974 - June 30, 1975

This office with the included Virus Studies Section and two separate electron microscopy units continues to be involved in a number of projects concerned with viral ultrastructure, viral replication and oncogenic virus-host cell interactions. These projects are as follows:

A. A search for viruses in biopsies and tissue culture isolates of human solid tumors and other human and other primate tissues.

B. Ultrastructural characterization of oncogenic and related viruses.

C. Ultrastructural, biochemical and immunological analysis of viruses of guinea pigs.

D. Ultrastructural and biochemical studies on viral RNAs.

E. Ultrastructural, histochemical and biochemical changes in cells during transformation following Rous sarcoma virus infection.

F. Details of the function of the nucleolus in transcription of the RSV genome and of the intracellular maturation and assembly of the virion.

A. Search for Viruses

Studies of tissue culture isolates from four human placentas have been examined with the electron microscope with negative results. Five new monolayer cultures obtain from biopsies of human solid tumors are presently being studied. Five suspension cultures were lost through contamination, probably from the use of contaminated media. Surviving cell lines are being stored in liquid nitrogen, once it has been determined that they can be regrown from the frozen state. These lines will thus be available to investigators interested in further characterizing them.

B. Ultrastructural Characterization

Ultrastructural identification of intracytoplasmic A particles and budding and mature type B particles in a baboon prostate indicates that the type B as well as the type C viral genome is present in as high a primate as the baboon. A herpes type virus distinct from HSV and EBV has been found in a tissue culture isolate from a Hodgkin's disease patient. Two other cell lines from Hodgkin's patients were found to contain type C particles in small numbers.

R type intracisternal particles have been identified in a tissue culture isolate from a human tumor. In this case there has been no possibility

of contamination with either hamster or collared lemming cells - the only previous species in which R type particles have been found.

Studies on the fixation of viruses have demonstrated the importance of the fixatives used, the length of fixation, whether fixed in vivo or in vitro and location of the virions in pellets in determining the final results. These variables apply to the preservation of both DNA and RNA viruses.

Under the auspices of the Viral Cancer Program a very successful symposium on the Characterization of Oncorna and Related Viruses was held at the Shoreham-Americana Hotel, Washington, D. C., Sept. 22-24, 1974.

C. Guinea Pig Viruses

A virus induced to replicate in guinea pig cells by exposure to halogenated pyrimidines has been shown to be closely related to the mouse mammary tumor virus. This close relationship has been demonstrated by similarity in ultrastructure and mode of replication, by identical density determinations, by the presence of high molecular weight RNA and of a reverse transcriptase preferring Mg^{++} instead of Mn^{++} in the reaction mixture.

An intracisternal particle is present in guinea pig leukemia cells. It differs from typical intracisternal A particles in possessing a distinct intermediate or capsid layer. In this respect it is more like a type C particle than an intracisternal A particle, even though it buds from the endoplasmic reticulum. These particles are found in fairly large numbers in the plasma of guinea pigs with terminal leukemia. They apparently appear in the plasma following their release from disrupted leukemic cells. Studies are under way which are attempting to demonstrate what relationship, if any, exists between the intracisternal and the type B particles.

D. Viral RNAs

Ultrastructural and biochemical studies have been carried out on the RNAs of 6 oncorna viruses and of vesicular stomatitis and Newcastle disease viruses and of the 28s ribosomal RNA from guinea pig cells. The results indicate that the subunit nature of oncornavirus RNA is universal and that it consists of a number of smaller molecules ranging in length from 0.3 μ to 2.2 μ corresponding to 0.4 to 2.6 x 10^6 daltons. Present in the high molecular weight RNA material was less than 1% of double-stranded DNA whose origin and significance is unknown. It is clear, however, that these DNA molecules were previously identified as viral RNA.

E. Viral Transformation

Vacuoles develop in chick embryo cells during transformation following RSV-BH infection. Using a mutant non-permissive of transformation at 41° C but permissive at 38 C, it has been possible to control vacuole formation and therefore to study it in detail. These vacuoles serve as markers for transformation. They apparently contain mostly water but may be involved in ion transport. Future studies will investigate this possibility.

F. Nucleolus in Transcription

Preliminary results of exposure of chick embryo cells to a-amanitin, a specific inhibitor of a RNA polymerase B, have shown changes in the nucleolus and endoplasmic reticulum apparently associated with the induction of immature intracytoplasmic A particles.

Electron Microscopic Technique

Electron microscope preparations for the office including Dr. K. Perk, 568 specimens fixed, embedded, sectioned, mounted and stained.

Photography

For the Office:

Routine prints	3,270
Plates for publication	17
Projection slides:	
2 x 2	600
3-1/4 x 4	50
4 x 5 negatives	400

Special printing and/or slide projects:

Drs. Moloney, Chirigos, Boone, Andrese, Holder, J.W. Pearson, Shelton, Sibal and Gruber. For J. W. Pearson, autopsy photographs plus black and white and color slides.

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of the Coordinator for Ultrastructural Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electron microscopic studies on oncogenic viruses and on host cells

Previous Serial Number: Same

Principal Investigator: Dr. Albert J. Dalton

Other Investigators: Dr. Ursula Heine
Dr. J. E. Dahlberg
Dr. K. Perk
Dr. D. V. Ablashi
Dr. A. Hellman
Dr. S. S. Kalter

Cooperating Units:

Inside NIH
Primate Virus Section, VLLB, NCI
Biohazards Research Section, NCI

Outside NIH
St. Joseph's Hospital, Tampa, Florida
Southwest Foundation for Research and Education
San Antonio, Texas

Man Years:

Total	2.0
Professional	1.0
Other	1.0

Project Description

Objectives:

(A) To study tissue culture isolates of human solid tumors and other human as well as other primate tissues for the presence of candidate oncogenic viruses.

Methods Employed:

The same methods of preparing materials for electron microscopic analysis are used here, at St. Joseph's Hospital, Tampa, Fla. and at the Southwest Foundation for Research and Education, San Antonio, Texas so that the results can be compared directly. The same basic tissue culture techniques and media are also used in all three places.

Major Findings:

(A) The new human tissue culture cell lines have been developed during the past year, 5 as monolayers, 5 as suspension cultures. These cell lines are presently being analysed by electron microscopy. In some of the suspension cultures as well as in some of the earlier developed suspension cultures, numerous small vesicles or micorvesicles have been found. These microvesicles have been described earlier in human breast tissue and in the lumen of the acini of other glands as well as in association with human lymphoblastoid cell lines. They have been called "virus-like" and have actually been confused with viruses. In a study of these microvesicles it has been determined that they are the product of the lysis or partial lysis of epithelial cells and one of their origins is from the cristae of degenerating mitochondria.

(B) In collaboration with Dr. S. Kalter, Southwest Foundation for Research and Education, evidence for the presence and replication of a typical type B RNA virus in a baboon prostate previously inoculated with a carcinogen has been obtained.

The evidence consists of finding intracytoplasmic type A particles in prostatic epithelial cells, budding of typical type B particles with surface spikes and mature (old) type B particles with eccentric nucleoids, distinct intermediate layers and surface spikes in the lumen of the acini.

(C) Detailed studies of fixation of several type C virions (ESP-1, Moloney and Rauscher strains of murine leukemia virus) have indicated that in fixation procedures there are several parameters which are of prime importance. In comparing the ultrastructure of type C virions it is essential (1) that in vivo material be compared with in vivo material and that in vitro material be compared with in vitro material; (2) that the fixative and fixation time be identical; (3) that if glutaraldehyde is used as the fixative, the fixation time should be a minimum of 24 hours; and (4) that if monolayer or suspension cultures are fixed as pellets after centrifugation, particles budding from cells should be compared with each other only when the cells involved are at least three cell layers in from the surface of the pellets. These requirements are based on two observations. The first is that ultrastructural changes may continue to occur during at least the first twelve hours of fixation in glutaraldehyde. The second is that cells exposed directly to chemical fixation without protection from surrounding cells or protein are altered ultrastructurally when compared with cells

so protected. This observation also holds for cells and tissues fixed for studies by light microscopy.

Significance to Biomedical Research and the Program of the Institute:

(A) The development of these human tissue culture cell lines has a broader significance than their value in the search for human oncogenic viruses. Dr. J. Fogh at Sloan-Kettering Institute for Cancer Research has been given a number of these lines for his own research and has indicated that this number (18) of human tissue culture cell lines is unique. In addition some of these lines were used to determine the origin and lack of relationship to viruses of the microvesicles.

(B) It is of considerable significance that, through the ultrastructural visualization of replication of a type B oncorna virus, the existence of the type B oncorna virus genome has been demonstrated without question in as high a primate as the baboon.

Proposed Course:

In consideration of the retirement of the principal investigator not later than November 1975, arrangements are being made to store the established human tissue culture cell lines in liquid nitrogen for the future use of others. Collaboration with St. Joseph's Hospital and the Southwest Foundation for Research and Education will continue at least until late summer. An atlas on the ultrastructure of insect and plant viruses in collaboration with Drs. F. Haguenau and K. Maramorosch is still under development. It is hoped that all of the work preliminary to publication will be completed. The 7th in the series of monographs on "Ultrastructure in Biological Systems" "Ultrastructure of the Anterior Pituitary" will be ready for distribution March 14, 1975.

Honors and Awards:

Three lectures on the ultrastructure of oncorna viruses were given during the past year, two at the Dept. of Anatomy, University of Washington, Seattle and one at Howard University, Washington, D. C.

On Feb. 28, I chaired and participated in a seminar on oncorna and related viruses at St. Joseph's Hospital, University of South Florida, Tampa, Fla.

On May 2, I will chair a "Symposium on oncogenic viruses" sponsored by The New York Society of Electron Microscopists.

With Dr. U. Heine and Dr. J. L. Melnick, I organized and took part in a Symposium on the Characterization of oncorna and related viruses, Shoreham-American Hotel, Washington, D. C., Sept. 22-24.

Consultant in Electron Microscopy, M. D. Anderson Hospital and Tumor Institute.

Member of the Scientific Advisory Committee of the Institute for Medical Research, Camden, N.J.

Member of the N.I.H. Florence Agreement Committee (electron microscopes and ultramicrotomes).

Publications:

Dalton, A.J., Hellman, A., Kalter, S.S. and Helmke, R.J.: Ultrastructural comparison of placental virus with several type C oncogenic viruses. J. Natl. Cancer Inst. 52: 1379-1381, 1974

Dalton, A.J., Haguenau, F., co-editors of monograph #7 in Ultrastructure in Biological Systems. The Ultrastructure of the Anterior Pituitary. Primary editors, M.G. Farquhar and M. Tixier-Vidal, March 1974. Academic Press, New York and London.

Dahlberg, J.E., Perk, K. and Dalton, A.J. Virus-like particles induced in guinea pig cells by 5-bromo -2' deoxyuridine are morphologically similar to murine B type virus. Nature 249: 828-830, 1974.

Dalton, A.J. Microvesicles and vesicles of multivesicular bodies vs "Virus-like" particles. J. Natl. Cancer Inst. (in press).

Dalton, A.J., Heine, U.I. and Melnick, J.L. Report on a symposium on "The characterization of oncorna and related viruses". J.Natl. Cancer Inst. (In press).

Dalton, A.J., Melnick, J.L., Bauer, H., Beaudreau, G., Bentvelzen, P., Bolognesi, D., Gallo, R., Graffi, A., Haguenau, F., Heston, W., Huebner, R., Todaro, G. and Heine, U.I. The case for a family of reverse transcription viruses: Retraviruses. Intervirology (In press).

1. Office of the Associate Director, Viral Oncology Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on virus-host cell interactions

Previous Serial Number: Same

Principal Investigator: Dr. Ursula Heine

Other Investigators: Dr. G. S. Beaudreau
Dr. C. F. Garon
Dr. G. H. Weber
Dr. P. Zamecnik

Cooperating Units: Inside NIH
Laboratory of Biology of Viruses, NIAID

Outside NIH
Oregon State University, Department of Agricultural Chemistry, Corvallis, Oregon
Harvard University, Mass. General Hospital Boston, Mass.

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description

Objectives:

(A) To study the relationship of selected tumor viruses to their host cells.

(B) To investigate variations in fine structure of different tumor viruses.

(C) To study the genome of RNA tumor viruses by biochemical and ultrastructural methods.

Methods Employed:

(A) and (B) Virologic methods and electron microscope techniques including cryosectioning for cell studies and studies of the fine structure of tumor viruses.

(C) The following methods are employed to study the viral genome: RNA and RNP extractions, gradient sedimentations, spectrophotometry, electron microscopy including the Kleinschmidt monolayer technique, positive, negative staining, and autoradiography at high resolution.

Major Findings:

(A) Three established cell lines from biopsies of patients with Hodgkin's disease were repeatedly screened for the presence of known tumor viruses. Recently, a herpes type virus was observed in one of the cell lines (single, floating cells). Biochemical investigations provide evidence for differences in the composition of its viral DNA when compared with DNA of HSV or EBV.

Two cell lines growing as monolayers in an epithelioid pattern contain C type virus in very low numbers. Cellular transplants of these cultures into nude mice produced malignant tumors. This study is done in collaboration with Dr. P. Zamecnik. A publication is in preparation.

(B) Previously, we have found that the appearance of DNA containing viruses of the herpes type can be changed drastically by employing different routine fixatives. Reported discrepancies in the fine structure of these viruses could thus be explained. Further investigations in this direction revealed that the ultrastructural appearance of the virions, especially that of the viral cores, is also highly dependent on the age of the virions and morphological details could best be observed in immature cores inside the infected nuclei. Moreover, this investigation also established morphological differences among the cores of herpesviruses of different origin when prepared for electron microscopic observation in identical manner. These findings can be used as a basis for differentiation of these virions. A publication of the results is in press.

(C) In collaboration with Dr. G. H. Weber (NCI - 4815), the genomes of six oncornaviruses were examined in the electron microscope by using a modification of the Kleinschmidt technique which permits the visualization of single stranded nucleic acids without breakage of covalent bonds. All genomes (60-70 S high molecular weight RNA) studied are composed of short molecules ranging in length between 0.3 μ and 2.0 μ. The larger molecules of 2.0 μ correspond to a molecular weight of 2.6×10^6. A model of the viral genome is proposed.

The genomes of vesicular stomatitis virus and Newcastle disease virus and the 28 S ribosomal RNA from guinea pig cells were used as controls. Their length determination showed that the length of the molecules corresponds

well with their molecular weight, thus degradation during preparation of the molecules under study is not likely.

In addition, it was found that all preparations of 60-70 S oncornavirus RNA contain a small number of double-stranded DNA molecules of unknown origin. The length of these DNA molecules is between 6 and 12 μ.

Significance to Biomedical Research and the Program of the Institute:

(A) C-type viruses are known to be the causative agents of many tumors in different animals including, as recent studies have shown, primates. Herpes type viruses are also closely associated with numerous tumors. It is important to establish a causative relationship between these viruses and human malignancies which, in the future, may lead to treatment of the disease by attacking the virus by chemotherapeutic means or by developing vaccines.

(B) and (C) The clarification of the fine structure of tumor viruses and the characterization of their components is of importance for the understanding of infection and the interaction of host genome with viral genome.

Proposed Course:

The study of the fine structure of different viruses will be continued and cryosectioning will be introduced to investigate viral micromorphology in an unfixed state.

The viral genome of oncornaviruses, especially the 30-40 S subunits, will be further investigated to detect possible degradation products. Statistical methods and the study of molecules radioactively labelled at specific sides will be used to help solve this question.

Honors and Awards:

Dr. Heine was invited to present the following paper "Electron microscopic observations on the composition of herpestype virions" at the 2nd International Symposium on "Oncogenesis and Herpesviruses", Nuremberg, Germany, Oct. 1974.

Presented the paper "Visualization of single-stranded RNA tumor virus nucleic acid with the electron microscope" at the XI International Cancer Congress, Florence, Italy, Oct. 1974.

Presented the lecture "The visualization of oncorna virus RNA in the electron microscope" at the Medical School in Hanover, Germany, Oct. 1974.

Acted as co-chairman at the symposium on "Classification of oncorna and related viruses", Washington, D. C., Sept. 1974.

Dr. Heine is invited to preside as co-chairman of the Virology Session, Am. Meeting of the AACR, San Diego, May 1975.

Publications:

Heine, U. and Ablashi, D.V.: Morphological studies of Herpesvirus saimiri in primate cell cultures. J. Med. Primatology, 3: 18-26, 1974.

Heine, U. and Dalton, A.J.: Ultrastructural analysis of herpes type viruses. In: Molecular Studies in Viral Neoplasia, The Williams and Wilkins Co., Baltimore, Md. 1974, 63-96.

Heine, U.: Intranuclear Viruses. In: The Cell Nucleus, Vol. III, In Busch, H. (ed.): New York, Academic Press, Inc., 1974, pp. 489-536.

Heine, U. and Cottler-Fox, M.: Electron Microscopic Observations on the composition of herpes type virions. In: "Oncogenesis and Herpesviruses" (In press).

Heine, U.I., Cottler-Fox, M., and Weber, G.H.: Visualization of tumor virus RNA in the electron microscope. In: Methods in Cancer Research, In Busch, H. (ed.): New York, Academic Press (In press).

Dunkel, V.C., Bast, Jr., R.C., Gerwin, B.I., Heine, I., Cottler-Fox, M. and Borsos, T.: Brief Communication: Presence of A-type and absence of C-type virus particles in a chronically induced guinea pig hepatoma. J. Natl. Cancer Inst. 53: 591-593, 1974.

Weber, G.H., Heine, U., Cottler-Fox, M., and Beaudreau, G.S.: Visualization of single-stranded nucleic acid of RNA tumor virus with the electron microscope. Proc. Natl. Acad. Sci. USA, 71: 1887-1890, 1974.

Weber, G.H., Dahlberg, J.E., Cottler-Fox, M., and Heine, U.: Electron microscopy of single-stranded RNA from vesicular stomatitis virus. Virology 62: 284-287, 1974.

Weber, G.H., Heine, U., Cottler-Fox, M., and Beaudreau, G.S.: Direct visualization of labeled nucleotides in DNA using electron microscopy and autoradiography. J. Cell Biol. 61: 257-261, 1974.

Weber, G.H., Heine, U., Cottler-Fox, M., Garon, C.F., and Beaudreau, G.S.: Nucleic acids of RNA tumor viruses: Identification and ultrastructure. Virology, (In press).

Ablashi, D.V., Loeb, W.F., Pearson, G., Valerio, M.G., Armstrong, G.R., Cicmanec, J.L., Heine, U., and Kingsbury, E.W.: Comparison of lymphomas induced in owl monkeys with Herpesvirus saimiri (HVS), heated HVS, and HVS-genome carrying cells. In: Multiple Primary Malignant Tumors. In Severi, L. (ed): Perugia, Italy, University of Perugia, (In press).

Serial No. Z01-CP-04814-04-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural studies concerning the role of cellular organelles in oncornavirus infection and in virion replication.

Previous Serial Number: Same

Principal Investigator: Dr. R. Gerald Suskind

Other Investigators: Dr. Francoise Haguenau
Dr. Giancarlo Rabotti
Dr. Susan C. Michelson-Fiske

Cooperating Units: Inside NIH
None

Outside NIH
Laboratoire de Medecine Experimentale
College de France, Paris

Man Years:

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description

Objectives:

Elucidation of the function of the nucleolus with respect to the regulation of transcription of the viral genome and induction of the transformed state. Clarification of the ultrastructural sites of transcription of the viral genome of Rous sarcoma virus and determination of the precursor sites of virion associated RNA and DNA. Search for cytochemical indicators of the template activity of chromatin. Morphologic clarification of the intracellular maturation and assembly of the virion.

Search for and study of viruses associated with human tumors.

Methods Employed:

The main techniques employed are quantitative electron microscopic and light microscopic autoradiography of tritiated RNA and DNA precursors in tissue culture cells exposed to various metabolic inhibitors and statistical evaluation of grain counts per unit area of organelle with the aid of planimetry. This requires growth of individual leukosis-free chick embryo cultures in serial passage and selection, freezing and storage of those susceptible to total transformation by the SR strain of RSV; electron-microscopic screening for C-particles and mycoplasma; the cloning, plaque purification and titration of virus pools, interference titration; assay of thermo-sensitive mutants; electron microscopic and cytochemical staining techniques.

Major Findings:

Results of previously outlined autoradiographic studies concerning nucleolus associated RNA and DNA synthesis in early stages of infection in Rous sarcoma virus infected chicken fibroblasts have been published. An amplification of the binding sites of Actinomycin to nucleolar chromatin in infected cells and a relative increase in nucleolar DNA synthesis were found to be correlated with increased nucleolar RNA synthesis at a very early period of infection. The relevance of these results to the induction of the transformed state is under investigation with thermo sensitive mutants of RSV. Results so far obtained are inconclusive other than to the instability of these mutants. Other studies, using polyacrylamide gel electrophoresis to characterize the nucleolar RNA synthesized in transformed cells recovering from inhibition by Actinomycin are in progress in collaboration with Dr. Rabotti, but are still in a preliminary stage. Results so far have not indicated the presence of viral RNA. Continuing autoradiographic experiments to clarify the source of virion associated DNA point both to a cytoplasmic and nucleolar precursor site of this DNA.

Exploratory experiments on the ultrastructural localization of the DNA binding dye Acridine Orange to nuclei of RSV transformed cells have shown some alteration in the localization of dye complexes. The feasibility of developing and applying this method into a quantitative technique, that may have relevance to the template activity of chromatin, is under investigation. Related experiments have been conducted on the labeling of DNA with tritiated Actinomycin in fixed transformed cells. Specific labeling has been observed sporadically by E.M. autoradiography, but standardization has not been achieved so far.

In order to study cellular aspects of virus maturation and transcription autoradiographic and electron microscopic studies are being conducted with a-amanitin, a specific inhibitor of a RNA polymerase B located in the nucleoplasm. Preliminary results have shown ultrastructural changes in the nucleolus and endoplasmic reticulum that appear to be associated with the induction of immature intracytoplasmic A particles.

A collaborative study with Dr. A. Hollinshead, G.W. University School of Medicine, has been initiated. This involves the electron microscopic study and characterization of a virus isolated from a human tumor stated to be of the herpes type. The presence of an intracisternal virus-like particle has been observed by electron microscopy of tissue cultures of this tumor.

Significance to Biomedical Research and the Program of the Institute:

The results of autoradiographic experiment can be interpreted as evidence for the activation of nucleolar templates of ribosome synthesis within one to two hours of infection with an avian oncornavirus. They constitute one of the earliest cellular reactions to infection with an oncogenic virus and point to the nucleolus as a likely site of derepression of "oncogenetic" DNA.

Proposed Course:

The characterization of an intracisternal virus-like particle associated with a human tumor will be pursued with inducing agents, biochemical isolation procedures, and electron microscopy.

The morphologic characterization of a herpes-like agent with neurotic properties associated with a human tumor will be attempted in a homologous and heterologous tissue culture system.

Autoradiographic and cytochemical studies relating to the genetic expression of chromatin in early stages of RSV infection, using tritiated Actinomycin and Acridine Orange as probes of DNA, will be conducted.

The cellular contributions to virion assembly in avian oncornavirus infection will be studied morphologically with inhibitors of RNA polymerase and ribosome synthesis.

Autoradiographic studies on the precursor of virion associated DNA will be pursued with inhibitors of DNA synthesis. In situ hybridization will be attempted in order to characterize the site of transcription of the viral genome.

Honors and Awards:

None

Publications:

Suskind, R. G., Michelson-Fiske, S., Haguenau, F., Rabotti, G. F.: A quantitative autoradiographic study of nucleolus associated RNA and DNA synthesis during the eclipse phase in Rous sarcoma virus infected chicken fibroblasts. J. Natl. Cancer Inst., in press.

Serial No. ZO1-CP-04815-02-ADVO

1. Office of the Associate Director, Viral Oncology Division of Cancer Cause and Prevention
2. Office of the Coordinator for Ultrastructural Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Nucleic acids associated with RNA tumor virus replication

Previous Serial Number: Same

Principal Investigator: Dr. George H. Weber

Other Investigators: Dr. U. Heine
Dr. G. S. Beaudreau
Dr. C. F. Garon
Dr. R. A. Lazzarini
Dr. J. E. Dahlberg

Cooperating Units:

Inside NIH
Laboratory of Biology of Viruses, NIAID
Laboratory of Molecular Biology, NINDS

Outside NIH
Oregon State University, Department of Agricultural Chemistry, Corvallis, Oregon

Man Years:

Total:	1.5
Professional:	1.0
Others:	0.5

Project Description

Objectives:

To study the structure, replication and interrelationship of the nucleic acids of RNA tumor viruses.

Methods Employed:

Ultrastructural analysis of purified nucleic acids by modifications of the Kleinschmidt spreading technique coupled with techniques for autoradiography of isolated nucleic acid strands.

Major Findings:

The RNA genome of a number of oncornaviruses has been examined with the electron microscope with the results that previous work on the subunit nature of oncornavirus RNA appears to be universal. All preparations of high molecular weight RNA isolated from MC29, AMV, RLV, RSV-Pr, RD114 and MLV consist of a number of smaller molecules ranging in size from 0.3 u to 2.2 u corresponding to 0.4 to 2.6 x 10^6 daltons. Extensive studies on the high molecular weight nucleic acids revealed the presence of DNA having length characteristics of 6-12 u. Present in relatively small amounts (less than 1%) this double-stranded DNA has been found in virtually all preparations of 60-70 S oncornavirus RNA. Its origin is unknown, although it is assumed to be cellular. This work was done in collaboration with Dr. G. S. Beaudreau and Dr. C. F. Garon. A publication describing this work is in press.

In a continuing study on the ultrastructure of the RNA genome of vesicular stomatitis virus (VSV), we have established that the linear, non-segmented RNA of VSV serves as an excellent control molecule for RNA electron microscopic studies. Preparations of 40-45 S RNA, as seen with the electron microscope, result in a narrow length distribution, characteristic for intact, homogeneous, and non-degraded RNA. The presence of smaller molecules in the 40-45 S preparation has been shown to be due, at least in part, to smaller defective virus. The work was done in collaboration with Dr. J. E. Dahlberg and has recently been published.

In collaboration with Dr. R. A. Lazzarini, studies are continuing on the ultrastructure and replication of the RNA genome of VSV. Preliminary results with defective virus have identified the presence of viral RNA molecules being composed of message and anti-message (genomic) arranged in a covalently-linked, double-stranded hairpin structure. A publication is in preparation.

Significance to Biomedical Research and the Program of the Institute:

Clarifying the role of RNA tumor virus nucleic acid replication and its association with host cell chromatin is important in deciphering the steps involved in transformation of infected cells. A direct observation of virus specific nucleic acids within host cell DNA would lend further evidence to the viral etiology of malignant transformation by RNA tumor viruses.

Proposed Course:

Methods are now underway to determine whether the small molecules of the oncornavirus genome are specific or non-specific degradation products or whether they represent true subunits. In addition, examinations into the in vitro replication of RNA tumor viruses will be expanded to include the detection and interaction of proviral DNA with that of the host cell. Autoradiographic techniques have been established to complement these findings.

Honors and Awards:

Invited speaker at the Armed Forces Institute of Pathology, August, 1974.

Invited speaker at the symposium on "The Characterization of Oncorna and Related Viruses". Under auspices of the Virus Cancer Program, N.C.I. held Sept. 22-24, 1974, Washington, D. C.

Invited speaker at the Laboratory of Molecular Biology, National Institute of Neurological Diseases and Stroke, January 1975.

Invited speaker at Georgetown University School of Medicine, Department of Biochemistry, February 1975.

Invited speaker at the 2nd International Symposium on Autoradiography. Under the auspices of the Royal Microscopical Society to be held in July 1975 at the University of Edinburgh, Edinburgh, Scotland.

Publications:

Weber, G.H., Heine, U., Cottler-Fox, M. and Beaudreau, G.S.: Visualization of single-stranded nucleic acid of RNA tumor virus with the electron microscope. Proc. Nat. Acad. Sci. U.S.A. 71: 1887-1890, 1974.

Weber, G.H., Dahlberg, J.E., Cottler-Fox, M. and Heine, U.: Electron microscopy of single-stranded RNA from vesicular stomatitis virus. Virology 62: 284-287, 1974.

Weber, G.H., Heine, U., Cottler-Fox, M., Garon, C.F. and Beaudreau, G.S. Nucleic acids of RNA tumor viruses: identification and ultrastructure. Virology (In press).

1. Office of the Associate Director, Viral Oncology Division of Cancer Cause and Prevention
2. Office of the Coordinator for Ultrastructural Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: (A) Studies of the morphological and biochemical changes induced in CE cells during transformation with Rous sarcoma virus.
(B) Studies of alkaline phosphatase levels in cells transformed by Rous sarcoma virus.
(C) Determination of the total molecular weight of RNA within oncornavirus virions.

Previous Serial Number: Same

Principal Investigator: Dr. Artrice V. Bader

Other Investigators: Dr. John P. Bader

Cooperating Units: Inside NIH

Cell Growth Regulation Section
Chemistry Branch, NCI

Man Years:

Total:	2.5
Professional:	1.0
Others:	1.5

Project Description

Objectives:

(A) To determine the nature of the vacuoles which typify transformation of CE cells infected with the Bryan "high titer" strain of Rous sarcoma virus.

(B) To study biochemical changes occurring in cells transformed by Rous sarcoma virus, which may be associated with the vacuolization occurring in these cells.

(C) To determine the precise molecular weight of native viral RNA extracted from oncornavirus virions.

Methods Employed:

(A) Tissue culture, phase contrast microscopy, cytochemical methods for electron microscopy, transmission electron microscopy.

(B) Tissue culture, phase contrast microscopy, enzymatic assays, spectrophotometric analysis.

(C) Transmission electron microscopy, quantitative particle counting techniques, polyacrylamide gel electrophoresis, tissue culture.

Major Findings:

(A) Initial phases of this project consisted of an investigation of the nature of vacuolization which accompanies the transformation of chick embryo cells by Rous sarcoma virus, Bryan "high titer" strain (RSV-BH). Phase contrast microscopy revealed highly refractile bodies varying in size and number appearing in the perinuclear region of the cytoplasm. Scanning electron microscopy revealed crater-like images developing in the cell surface suggesting that the vacuoles are composed of a volatile substance, probably water. Histological staining studies did not detect lipids, proteins, glycoproteins, polysaccharides or nucleic acids within the vacuoles. Transmission electron microscopy showed that these refractile bodies are membrane bound, intra-cellular components.

A virus mutant, RSV-BH-Ta, selected for a defect in the transformation region of the viral genome allowed a study of the development of vacuolization. Shifting mutant infected cells from the transformation non-permissive temperature (41°) to the transformation permissive temperature (37°) induces the formation of vacuoles. Phase contrast microscopy showed the development of vacuoles in the perinuclear region of the cell. Vacuoles induced during transformation can be distinguished from cellular phagocytic and pinocytic vacuoles.

Cytochemical methods combined with light and electron microscopy are being used to analyze vacuole synthesis and development within the cell. Experiments involving horse radish peroxidase labelling which utilizes an enzymatic reaction occurring at the cell surface are in progress to determine if the vacuoles occurring in these transformed cells are formed from their plasma membranes. Other cytochemical methods including the localization of ATPase are being used to determine if enzymatic activity is localized within the vacuoles or within vacuolar membranes. Localization of ATPase activities in these cells is important since physiological studies of the vacuoles suggest that Na^+ is an important factor in the vacuolization process. ATPases are involved in Na^+, K^+ transport as well as other cellular metabolic activities.

(B) Alkaline phosphatase levels in chick embryo cells were compared with cells transformed by Rous sarcoma virus. Cells infected with the Bryan

"high titer" strain (RSV-BH), a mutant (RSV-BH-Ta) inducing temperature dependent transformation and another virus strain, Schmidt-Ruppin (RSV-SR) were examined. Phosphatase activity was assayed using p-nitrophenyl phosphate as substrate. Chick embryo cells exhibited enzyme levels several fold higher than those of RSV-BH transformed cells. The mutant, RSV-BH-Ta, allows a non-transformed phenotype when infected cells are incubated at 41°, but at 37° the cells appear transformed. A comparison of alkaline phosphatase levels in cells grown at the two temperatures, showed that the cells at 41° had higher levels of enzyme than those grown at 37°. The differences were similar to those seen between CE cells and RSV-BH transformed cells. Observation of cells infected with RSV-BH-Ta and shifted from 41° to 37° showed that the decrease in enzyme level was a gradual change requiring between 18-24 hours for differences to be attained commensurate with differences found between non-transformed CE cells and RSV-BH transformed cells. Cells transformed by RSV-SR differ morphologically from those transformed by RSV-BH. Nonetheless, cells transformed by RSV-SR also contained lower alkaline phosphatase levels than CE cells. These results suggest that the decrease in alkaline phosphatase activity is a general property of transformed cells.

(C) A quantitative particle counting technique is being utilized to obtain an accurate reproducible counting system for oncornavirus virions. Virion aggregation imposes a probability of error in counting systems where a high level of sensitivity is required. Treatment by sonication was insufficient to completely disassociate aggregates as was exposure to a variety of enzymes. The general technical aspects of the procedure have been resolved.

Significance to Biomedical Research and the Program of the Institute:

(A) Biochemical and biophysical data pertaining to these vacuoles are compatible with morphological data. These combined results show an increase in intra-cellular water in cells transformed by RSV-BH and suggest a mechanism for malignant transformation by this virus based on water and ion exchange. A study of the vacuolization process may reveal basic physiological changes responsible for the malignancy of cells transformed by RSV-BH.

(B) Biochemical changes between transformed and non-transformed cells have been documented. A decrease in alkaline phosphatase levels in transformed cells possibly can be used as a biochemical marker to distinguish transformed cells from their normal counterparts.

(C) Because of the current investigations and controversies surrounding the molecular configuration of the viral RNA extracted from oncornavirus virions the reassessment of the molecular weight of this native RNA is significant at this time. An accurate molecular weight of oncornavirus RNA derived from highly purified vRNA will give needed data which will aid in the resolution of the problem of the existence or non-existence of sub-units of vRNA.

Proposed Course:

(A) Extension and completion of the cytochemical studies now in progress.

(B) Preliminary cytochemical alkaline phosphatase experiments were in progress at the time differences in enzymatic activity were found. The initiation of these studies was to determine if the enzyme was a constituent of the vacuoles of the RSV-BH transformed cells. Since the transformed cells have lower levels of enzyme than non-transformed cells, it now seems unlikely that this enzyme will be associated with the vacuoles.

(C) Other enzymatic treatments, separately and in conjunction with sonication will continue to be used. Once the counting technique becomes reproducible with this virus system, the molecular weight will be extrapolated based on the grams of RNA determined biochemically using agar gel electrophoresis and the moles of RNA determined by electron microscopy particle counting.

Honors and Awards:

Session Chairman, RNA Tumor Viruses, American Society for Microbiology, Annual meeting - Chicago, Illinois, May 1974.

Presented lecture "Virus production and cellular transformation by RNA-containing tumor viruses", Winston-Salem State University, Winston-Salem, North Carolina, September 1974.

Awarded National Research Council Travel Award to attend the XI International Cancer Congress, Florence, Italy, October 1974 and presented paper "Characteristic Vacuolization of Cells Transformed by Rous Sarcoma Virus".

Publications:

Bader, John P. and Bader, A.V.: Characteristics of cells transformed by Bryan Rous sarcoma virus in Mechanisms of Virus Disease, In Robinson, W.S. and Fox, C.F. (eds.): Menlo Park, Calif., W.A. Benjamin, Inc. 1974, pp. 303-313.

Serial No. Z01-CP-04818-02-ADVO

1. Office of the Associate Director, Viral Oncology Division of Cancer Cause and Prevention
2. Office of the Coordinator for Ultrastructural Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: (A) Morphological and biochemical characterization of the BrdU-induced B-type guinea pig virus.
(B) Characterization of the virus-like particles present in the L2C transplantable guinea pig leukemia.
(C) Morphological comparison of the intracisternal viruses of several mammalian species.

Previous Serial Number: Same

Principal Investigator: Dr. John E. Dahlberg

Other Investigators: Dr. Kalman Perk
Dr. R. Michalides
Dr. A. J. Dalton
Dr. J. Schlom
Dr. J. Pearson

Cooperating Units:

Inside NIH
None

Outside NIH
Flow Laboratories
Meloy Laboratories

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

(A) To substantiate by biochemical and immunological methods our earlier morphological findings which indicated that the BrdU- inducible guinea pig virus was very similar to the murine mammary tumor virus but not to C-type viruses.

(B) To characterize morphologically, and as far as possible, by biochemical and immunological means, the intracisternal virus present in guinea pig L2C tumor cells, and to compare this virus with the BrdU-inducible guinea pig virus and also to intracisternal viruses occurring in other species.

(C) As an adjunct to (B), intracisternal viruses, and viruses capable of growing in the endoplasmic reticulum, will be analysed morphologically in order to determine what features they have in common and in what ways these virus particles can be grouped based on differences in structure and morphogenesis.

Methods employed:

Light and electron microscopy, cell culture, virus growth and purification, ultracentrifugation, and other preparative and analytical procedures.

Major Findings:

(A) In collaboration with Drs. K. Perk, R. Michalides, and J. Schlom. The morphological properties of the virus particles released from guinea pig cells following induction with halogenated pyrimidines were shown to closely resemble those of the B-type murine mammary tumor virus, MMTV, while the guinea pig virus could be distinguished from mammalian C-type viruses in several ways. Since this is the first reported instance of a B-type virus occurring in an animal other than the mouse, substantial efforts were made to correlate the biophysical and biochemical properties of the BrdU-inducible guinea pig virus to those of MMTV.

When this guinea pig virus, grown in the presence of ^{3}H-uridine, is centrifuged to equilibrium in sucrose and cesium chloride, its density is 1.19 g/ml and 1.21 g/ml respectively. These values are identical to those for MMTV under identical conditions, and distinct from those of C-type viruses, which have a density of 1.16 g/ml in both solutes.

The BrdU-inducible guinea pig virus contains high molecular weight RNA and a reverse transcriptase. After demonstrating that the RNA complex has an apparent molecular weight typical of oncornaviruses, the cation preference of the enzyme was investigated. It has been known for some time that MMTV prefers Mg++ ions while mammalian C-type viruses prefer Mn++ in a reverse transcriptase reaction mixture. We have found that the cation preference of the GPV enzyme is identical to that of MMTV.

Based on these biophysical and biochemical similarities, it has been concluded that for all criteria tested, the BrdU-inducible GPV is closely related to MMTV.

(B) In collaboration with Drs. K. Perk and J. Pearson.
Efforts to characterize the intracisternal virus-like particle found in L2C guinea pig tumor cells have been continued. They differ from murine intracisternal A particles in several ways. Most important is the ability of the guinea pig intracisternal particles to be released from the dying tumor cells which may be present in a variety of tissues of the leukemic guinea pigs. Thin sections of high speed pellets of the plasma of leukemic guinea pigs reveal large numbers of virus particles. These particles appear to be identical to the extracellular particles present in the tissues of these animals.

An effort was made to explain why guinea pig intracisternal particles could be released from broken cells while murine intracisternal A particles are not. An analysis of serial sections of tissues and tissue culture cells containing large numbers of murine intracisternal A particles revealed that at least 99% of these particles remain attached to the membrane of the endoplasmic reticulum, despite their appearing separated from the ER membrane about 13% of the time when random sections are analysed. This was interpreted to mean that even when cells are broken the murine A particles would tend to remain cell associated. Guinea pig intracisternal particles, while difficult to analyse with serial sections due to their relative rarity, appear attached less frequently in random sections. This could mean that a fraction of these particles are completely detached and would be released if the cell were to break open.

Currently, we are analysing the properties of a reverse transcriptase activity found in the plasma of leukemic guinea pigs. The biochemical properties of the enzyme are identical in every respect to those of the BrdU-induced guinea pig virus, and it appears that, in some unexplained way, the onset of the L2C leukemia, containing an intracisternal virus, may also trigger the expression of the B type guinea pig virus. As yet, however, there is no morphological evidence as to where the B-type virus originates within the leukemic animal, since intracytoplasmic A particles and budding B type virus has not been observed in the L2C tumor cells, despite an extensive search. Efforts to reconcile the morphological evidence with the biochemical data are being actively pursued.

(C) In collaboration with Drs. A. J. Dalton and K. Perk.
Intracisternal virus-like particles occur with regularity in a large variety of rodent and other mammalian species. In many cases they occur in large numbers in tumor cells, but they may also occur in normal cells. Their importance in tumor virology is largely unexplored, and one reason for this is the lack of a source of information which combines what is known about their ubiquity and morphological similarities with the more limited biophysical and biochemical data available. We are currently obtaining tissue and EM samples for observation and have accumulated a large number of electron micrographs of most of the known intracisternal viruses.

Significance to Biomedical Research and the Program of the Institute.

(A) The major conclusion from these experiments is that B type viruses are not limited to mice. Furthermore, a variety of biochemical and biophysical criteria previously shown to be useful in distinguishing MMTV from mammalian C-type viruses are also useful in defining newly isolated viruses as B-type or C-type.

(B) We have been able to differentiate guinea pig intracisternal virus particles from murine intracisternal A particles by demonstrating that murine particles are much more likely to remain attached to the membrane of the endoplasmic reticulum and therefore are not able to be released from necrobiotic cells. The significance of this finding is uncertain at this time, but it is evident that a virus which remains cell associated at all times has little potential of being infectious.

We have demonstrated a reverse transcriptase activity in the plasma of leukemic guinea pigs. The properties of this enzyme indicate that the B-type BrdU-inducible GPV is probably present in leukemic guinea pigs, yet examination of leukemic guinea pigs by electron microscopy has failed to reveal any of the B-type GPV particles. It would appear that the leukemia either induces or releases small amounts of either the B-type virus or a precursor into the plasma, but a complete explanation is not yet available.

(C) This project is of value if it is able to focus attention on a largely neglected area of viral oncology. Very little is known about intracisternal virus-like particles, yet the available information suggests an intimate relationship between these particles and the transformed state.

Proposed Course:

(A) It has proven difficult to completely purify the BrdU-inducible GPV because of its tendency to aggregate. Efforts will be made to find means of alleviating this problem in order to unambiguously determine the identity of the viral proteins. This will permit comparison to both B-type and C-type viruses in order to determine if the size of viral proteins might also be an indicator of virus classification.

Antiserum to B-type guinea pig virus should soon be available. After testing against control material and suitable exhausting the sera to remove unwanted antibodies, the sera will be used to determine if there is any relatedness between the B-type GPV and the virus-like particles present in leukemic guinea pigs. It will also be fully tested against all available B- and C-type viruses to determine if there is any antigenic relatedness.

(B) We will attempt to explain how the B-type GPV appears in the plasma of leukemic guinea pigs without being apparent in the electron microscope. This will involve further exploration by EM as well as biochemical tests of leukemic plasmas to determine the fraction of virus antigen and RNA

which is specific to the B-type GPV. A low fraction could account for both the presence of B-type GPV and the apparent absence of morphologically identifiable virus.

(A) An analysis of the morphological features of intracisternal viruses will continue. It is already apparent that these viruses are a complex group and that an attempt at categorization of variation within this group could be of importance.

Honors and Awards:

Invited speaker at a symposium on "The characterization of oncorna and related viruses", Washington, D. C., Sept. 22-24, 1974.

Invited speaker at the Ninth Annual Joint Working Conference, The Virus Cancer Program, NCI, Hershey, Penna., Nov. 10-13, 1974.

Publications:

Dahlberg, J.E., Perk, K., and Dalton, A.J. Virus-like particles induced in guinea pig cells by 5-bromo- 2' -deoxyuridine are morphologically similar to murine B-type virus. Nature 249: 828-830, 1974.

Perk, K., and Dahlberg, J.E. (1974) Murine intracisternal A type particles fail to separate from the membrane of the endoplasmic reticulum. J. Virol. 14: 1304-1306, 1974.

Weber, G.H., Dahlberg, J.E., Cottler-Fox, M., and Heine, U. Electron microscopy of single-stranded RNA from vesicular stomatitis virus. Virology 62: 284-287, 1974.

Serial No. Z01-CP-04991-04-ADVO

1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention.
2. Office of the Coordinator for Ultrastructural Studies.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural studies on tissue and virus in relation to neoplastic diseases

Previous Serial Number: NCI-4874

Principal Investigator: Dr. Harish C. Chopra

Other Investigators: None

Cooperating Units: Inside NIH

Dr. G. Vlahakis, LBGY, NCI (NCI-3265)
Dr. N. Javadpour, SURG, NCI (NCI-3819)
Dr. A. Gazdar, VLLB, NCI (NCI-4824)
Dr. A. Ablashi, VLLB, NCI (NCI-4834)

Outside NIH

Dr. A. Gottlieb, Rutgers University, New Brunswick, New Jersey
Dr. Donald Fine, Frederick Cancer Research Center, Frederick, Maryland
Dr. Marcus Mason, Mason Research Institute, Worcester, Massachusetts.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(A) Study of virus-cell interaction during various developmental stages of mouse mammary tumors.

(B) Electron microscopic study of human mammary and renal carcinomas.

(C) Morphological study of dog mammary carcinomas for detection of virus particles.

(D) Collaborative participation with NCI investigators to provide electron microscopic support for various research projects.

(E) Completion of previously reported research projects.

Methods Employed:

The mammary gland plaques, nodules, and early as well as late mammary tumors from different strains of mice were processed for electron microscopy. Similarly human tumor biopsies and their tissue cultured cells were studied by thin section electron microscopy. Various research projects of collaborating investigators were discussed and the specimens submitted for electron microscopy were examined. The electron micrographs were interpreted, evaluated and prepared for publications.

Major Findings:

(A) The mammary gland plaques and early mammary tumors from GR and DD strains of mice, and mammary gland nodules of $C3H^{Avy}$ mice were investigated to study virus-cell interaction for in vivo transformation. The mammary gland plaques, considered as pregnancy dependent preneoplastic tumors, show certain common features with the mouse mammary tumors. It is observed that a group of mammary epithelial cells form small lobules with a central ductule in which secretory material is accumulated. A large number of extracellular virus particles are observed in these ductules. However very few particles were observed in the budding stages. The relationship of extracellular virus particles to the intracytoplasmic A particles, (the latter were much fewer) could not be established except that the inner double-shelled core of budding particles resembles the intracytoplasmic A particles. In the plaque cells both extracellular and intracytoplasmic virus particles were observed simultaneously. However, still very early stages of plaque formation are to be examined to investigate the role of virus particles in the transformation of mammary cells into plaques. No significant morphological changes were observed in the transformed plaque cells except prominent nucleoli, and enormous increase of ribonucleoprotein granules which morphologically appear to be the secretory product seen in the ductules. The plaques at later stages represent typical mammary tumors with larger ducts and an enormous number of prominent villi protruding from the cell surface. Apparently the mammary tumor cells in their late stages lose orientation around the ducts. The virus particles in the ducts are not found in clusters as seen in the plaques. The mammary tumor cells do not show any significant changes as compared to plaque cells except that they show a large number of villi on their cell surface. The nuclei consist of diffused chromatin and there may be an increase in number of mitochondria. There is also an increase of intracytoplasmic type A particles but very few budding particles were observed. Similarly the mammary gland nodules of $C3H^{Avy}$ a

preneoplastic condition which unlike the plaques are not pregnancy dependent, show common morphological features with mammary gland cells. Comparatively they show a much fewer number of virus particles and significantly, only rarely the extracellular mature type B particles with electron dense eccentric nucleoids were seen. Most often the particles in the intercellular spaces or in the ductules were observed as immature virus particles. The ductules observed in these mammary nodules were much fewer than those seen in the plaques or in the mammary tumors.

(B) We are continuing our interests in studying human tumors to identify virus or virus related morphological entities, especially to correlate with the biochemical marker RNA dependent DNA polymerase. The human breast carcinomas and renal carcinomas are being used for these investigations in view of the fact that these carcinomas are being cultured with greater success and the carcinoma epithelial cells are easy to maintain in cultures. We contemplate studying a large number of mammary carcinoma cultures in collaboration with the NCI breast clinic through which breast tumor material has now become available. Our special interest in studying the renal carcinomas is resulting from the recent reports of Elliot et al.(J. Natl. Cancer Inst. 53, 1341-1350, 1974) demonstrating the presence of "small virus-like" particles in renal carcinomas which were previously discovered by us in human breast tumor biopsies and in the milk of women with family history of breast cancer (J. Natl. Cancer Inst. 40: 1359-1373, 1968). Our preliminary studies have failed to reveal any virus-like morphological entities in these carcinoma cultures.

(C) A few more dog mammary carcinomas, making a total of 40 dog mammary tumors examined during the past three years, were received from the Mason Research Institute for electron microscopic search of virus particles. We had previously observed virus-like structures in three mammary carcinomas but our current studies failed to confirm such particles as virus particles. This project is being terminated and no further studies are being contemplated.

(D) We continue to undertake collaborative studies to provide electron microscopic support to other investigators in viral oncology. Dr. Gottlieb's project on mouse myeloma containing polymerase activity was investigated and it was demonstrated that intracisternal particles were present in abundance in this tumor tissue. These intracisternal particles are now being correlated with polymerase activity and infectivity. Similarly electron microscopic support was provided for Dr. Gazdar's project on infectivity of insect cells with mouse leukemia virus; and for Dr. Ablashi's rescue studies on antigenically (herpes) positive cells with chemical carcinogens. It is expected that a limited collaborative support will continue to be provided to viral oncology investigators.

(E) Various research projects reported previously were completed and reports compiled for publications.

Significance to Biomedical Research and the Program of the Institute.

Ultrastructural studies on cancer tissues and of oncogenic viruses in relation to neoplastic diseases have contributed significantly to the Virus Cancer Program. Special emphasis has been laid on the detection and isolation of viruses, resembling the known oncogenic animal viruses, in the human tumors or from the cell cultures established from the human neoplastic tissues. Further collaborative investigations with other NCI investigators have provided necessary support on ultrastructural aspects of various research projects.

Proposed Course:

It is planned to continue the above mentioned studies. However, emphasis will be placed on the correlation between virus-cell interaction and in vivo cell transformation. Also it is anticipated to undertake more collaborative studies with other NCI investigators.

Honors and Awards:

Invited to participate in a symposium on "The Characterization of Oncorna and Related Viruses" held September 22-24, 1974. Progressive developments on the virus 'Mason-Pfizer' isolated from monkey breast carcinoma were reported.

Invited to attend the 9th Meeting on Mammary Cancer in Experimental Animals and Man, October 28-30, 1974 in Pisa, Italy. Chopra, H.C.: Ultrastructural study of dog mammary tumors for detection and isolation of virus particles (abstract).

Publications:

Hall, W.T., Gazdar, A.F., Hobbs, B.A. and Chopra, H.C.: Morphology of a "noninfectious" sarcoma virus. J. Natl. Cancer Inst. 52: 1337-1343, 1974.

Ablashi, D.V., Armstrong, G.R., Easton, J.M., Adamson, R.H. and Chopra, H.C.: Evaluation of effects of Cytosine arabinosine on herpes virus saimiri replication in owl monkey kidney cells. Biomedicine 21: 57-60, 1974

Fine, D.L., Pienta, R.J., Malan, L.B., Kubicek, M.T., Bennett, D.G., Landon, J.C., Valerio, M.J., West, D.M., Fabrizio, D.A., and Chopra, H.C.: Biologic characteristics of transformed Mason-Pfizer monkey virus infected rhesus foreskin cells. J. Natl. Cancer Inst. 52: 1135-1142, 1974.

Fine, D.L., Landon, J.C., Pienta, R.J., Kubicek, M.T., Valerio, M.G., Loeb, W.F., and Chopra, H.C.: Responses of infant rhesus monkeys to inoculation with Mason-Pfizer monkey virus materials. J. Natl. Cancer Inst. (In press)

SUMMARY REPORT

c. OFFICE OF PROGRAM RESOURCES AND LOGISTICS

The Office of Program Resources and Logistics within the Office of the Associate Director for Viral Oncology is responsible for the review and scientific management of collaborative research contracts providing research resources and logistical support to intramural investigators within Viral Oncology and collaborating laboratories participating in the NCI Virus Cancer Program, and, when adequate resources are available, to the general scientific community for studies related to the possible viral etiology of human cancer. The Office is also responsible for the day-to-day general management and direction of resources distribution. The Office was established in 1972 to centralize the scientific administration and management of research resources and logistical functions and to unify these activities within the Office of the Associate Division Director.

Many of the research investigations carried out in the Viral Oncology Program depend on the availability of clinical and laboratory materials of optimal purity, viability, and potency. Studies in an integrated program of international scope, as encompassed in the VCP, make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures, and test animals are available. The Office of Program Resources and Logistics provides these and other supportive activities through contract operations representing six general areas of activities. These include:

1. Activities directed toward production and characterization of purified viruses and viral reagents.

2. Activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimen material.

3. Activities concerned with animal resources, including production of pathogen-free and germ-free species of animals, breeding of primates, maintenance of animal colonies, and containment-type holding facilities.

4. Activities directed toward the provision of specialized testing services for the examination of experimental materials.

5. Activities concerned with providing biohazard and environmental control support capability.

6. Activities directed toward providing laboratory facilities and services for direct support of NCI intramural research programs.

The overall requirement for research resources and the number and extent of requests received frequently exceed the availability of Program resources. This is due, to some extent, to the high cost of producing or preparing certain scarce reagents, to fiscal considerations which limit the ability to prepare every potentially necessary item, and to a valid reluctance to prepare highly specialized and expensive materials which can be utilized by only a few laboratories. Because requests do exceed availability, the distribution of resources is influenced by the relative value of specific research in relation to overall Program goals. These relative values, or priorities, are determined by the VCP joint Segment Chairmen, the VCP chartered Scientific Review Committees (peer review), the OPR&L Advisory Group, and the VO Branch and Associate Branch Chiefs. The distribution of resources is patterned on the evaluations and recommendations of these groups.

During the past year, this Office has coordinated the distribution of a wide variety of biological, chemical, and materiel resources to NCI intramural investigators and VCP participants. The amount of material processed has been enormous. To illustrate the scope of activity, during this year the purified and concentrated products from over 50,000 liters of tissue culture-grown viruses, propagated in over 35 different cell lines, were distributed in over 500 shipments to over 150 participating laboratories throughout the world. Additionally, approximately 500 grams of a single virus, not propagated in tissue culture, was sent to over 50 different investigators in over 100 shipments. Furthermore, the Program repositories handled or shipped out over 45,000 individual items. In addition to these activities, the Office has coordinated the distribution of a variety of resources to U.S.S.R. scientists in keeping with the Memorandums of Understanding signed in Moscow in 1972 and 1974 covering the mutual exchange of cancer research materials. The materiel supplied included purified and concentrated viruses of murine, feline, avian, and primate origin; specialized viral proteins and antigens; normal and infected tissue culture cell lines; various tumor-derived cells; a wide range of standard reagents and chemicals for tissue culture utilization, including dried powder media, radioisotope-labelled chemicals and nucleotides, and enzymes and hormones; a variety of antisera prepared in a wide range of host animals; various substances for chromatographic separations; adjuvants; surgical supplies; a specially ordered incubator-shaker; a laminar flow biological safety cabinet; and various other materials and substances.

An additional responsibility of OPR&L is the monitoring and evaluation of the virus production and purification activities of Viral Oncology at the NCI-Frederick Cancer Research Center. OPR&L is also responsible for the general management of the distribution of viruses, viral reagents and components, and cell cultures to FCRC intramural investigators and to VCP collaborating laboratories. During the period February 1974 to January 1975, over 11,000 liters of virus materials were produced and purified, and over 12,000 ml. of various virus concentrates were released to investigators as directed by this Office. All Program requests for viruses available from FCRC have been met and approximately 3,000 ml. of virus concentrates remain available at the FCRC virus repository to meet any additional needs.

The OPR&L is also concerned with the preparation of an annual catalog listing and describing the research resources available to collaborating laboratories within the Program. These include viruses, antisera, animal resources, special materials, and various services. Usually the information provided for each item includes designation, origin, and processing procedure. This year, as in prior years, a revised new edition of the catalog was prepared and distributed to Program participants. Loose-leaf binding, adapted earlier, was retained to permit efficient future updating of information, and the format and contents were again revised to expedite the recovery of items or services and reflect current research interests. As noted in the catalog, an element of the OPR&L is concerned with the maintenance of a computerized central inventory for the sera, tumor tissue, cell out-growths, and other human specimen materials continuously being acquired by the Program. The central inventory facilitates matching investigators' requests for human materials with specimens available, regardless of the geographic location of the repository or laboratory at which it is stored.

The Resources Data Management unit within OPR&L continued to assist in the automated retrieval and inventories of Virus Cancer Program resources, computer-systems planning consultation support, and automated resources analysis and management support. The automated inventories of VCP resources involve the OPR&L serum collection, the OPR&L human tissue collection, the serum collection developed and maintained for the NCI Office of Biohazards and Environmental Control, and the resources covered under the satellite local resource systems. The OPR&L RDM unit maintains a computerized inventory of approximately 150,000 human sera specimens and approximately 2,000 freshly frozen normal and malignant human tissue specimens maintained in five local and regional repositories. The RDM unit manages the continuous input, updating, and distribution data for these specimens stored for utilization by Program scientists. Additionally, donor, storage, and distribution data are maintained for sera specimens collected by the NCI Office of Biohazards and Environmental Control from research and related activity employees of Viral Oncology-supported contracts. Specimens are collected from individuals at the time of employment, annually, when injury or special illness occurs, during convalescence, and at the termination of employment. Furthermore, automated computer support is provided for previously developed and installed inventories in two laboratories participating as regional resource repositories for the VCP. These include the Cell Culture Laboratory of the University of California at the Naval Biomedical Research Laboratory, and also at the Huntingdon Research Center. Additionally, automated computer support is provided for the inventory system of the laboratory of the Deputy Clinical Director, NCI. Monthly inventory listings are provided for each of the satellite repositories maintained in the OPR&L automated inventory system, and additional *ad hoc* reports are prepared as required.

The OPR&L RDM unit has developed compatible automated systems and codes for human specimen material in all institutions participating in the VCP either as collection or acquisition laboratories, or as repository or distribution centers. This system includes the capture and automation of

complete demographic, clinical, and laboratory information on specimens and specimen donors. The goal has been a central specimen inventory at OPR&L, making available in a uniform manner for all of the VCP, the many specimens held at various repositories. Furthermore, all laboratories in the VCP are offered consultation in computer systems planning by the RDM unit of OPR&L so that VCP contractors can receive expert advice in developing, planning, and installing local automation systems for the managing and processing of experimental data, survey and statistical analysis, and available resources.

Additionally, the RDM unit is involved in the planning and implementation of an automated computer system for the capturing of data relevant to the distribution of research resources managed and administrated by OPR&L. Currently, efforts are in progress to continuously retrieve and update appropriate data relevant to the distribution of viruses and virus-related products. Following the development of the management system for virus reagents, it is anticipated that a comparable system will be developed for the management of human specimen material.

Within the Office of Program Resources & Logistics, principal support is provided by three staff scientists, a computer programmer, and two secretaries, who all assist in responsibilities for the management of the collaborative contract program concerning research resources, and for the coordination of resources distribution.

Serial No. ZO1-CP-04801-03-ADVO

1. Office of the Associate Director, Viral Oncology
2. Division of Cancer Cause and Prevention
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Electron Microscope Development and Studies of Viruses in Various Human and Animal Tissues

Previous Serial Number: 4927

Principal Investigator: Dr. Victor H. Zeve

Other Investigators: Dr. William J. Hamilton
Mr. Matthew A. Gonda
Mr. Kunio Nagashima

Cooperating Units: Inside NIH:

Dr. Stuart Aaronson, VCB, NCI
Dr. Dharam Ablashi, VLLB, NCI
Judith S. Prewitt, DCRT, OD/NIH

Outside NIH:

Dr. Donald Fine, LBI, FCRC
Dr. Larry Arthur, LBI, FCRC
Dr. Daniel Twardzik, LBI, FCRC
Dr. George Shibley, LBI, FCRC
Dr. Robert Nathan, J.P.L., Pasadena, California

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

(1) To develop new methods of utilizing both the transmission and scanning electron microscope as a quantitative analytical research tool.

a. To initiate a research program using the SEM and TEM coupled with energy-dispersive X-ray analysis and immunological methods of specimen preparation with the specific intent of identifying oncogenic viruses or subviral components.

b. To study the surface features of cultivated human tumor cell lines at the ultrastructural level with the SEM and TEM using new techniques of specimen preparation.

c. To develop rapid new methods of utilizing critical point dried virus preparations which have been immunochemically tagged.

d. Investigate the use of image intensification as a means of visualizing heavy metals in thin section materials in absence of conventional staining.

(2) To perform collaborative research with scientists in other areas of FCRC and NIH to investigate the effect of 5-tungsto-2 antimoniate (TA) on RNA virus producing cells.

Methods Employed:

Methods are being developed to utilize immunocytochemical techniques combined with SEM and automated X-ray energy spectroscopy (energy-dispersive analysis) to extend the capabilities of electron optical instrumentation to detect, localize, and quantitate viral and subviral entities. Reagent antisera prepared against whole oncogenic viruses and purified protein and glycoprotein subcomponents have been used to provide very good specificity of labeling in our immunochemical tagging methods. Ferritin and peroxidase conjugates have been used successfully in a two-stage "sandwich" labeling experiment and the unlabeled-antibody enzyme technique is in development. The heavy metal deposition given by the peroxidase staining techniques provide the mechanism for detection of the specifically labeled virus particles by the X-rays generated in the electron beam of the microscope. The methods are expected to increase sensitivity, specificity and speed of the current Automated Particle Analysis system for detection, localization and quantitation as well as in other applications.

A series of eight human tumor cell lines were compared in the SEM to equivalent normal types to study the ultrastructural surface features and social aspects of acquired cell malignancy in tissue culture.

A high resolution video camera capable of storage and integration was developed and installed on a transmission EM to electronically enhance contrast and digitize images for pattern recognition.

Reports in the literature indicated an in vivo effect of (TA) on the control of leukemia and sarcoma in mice. This compound was applied to in vitro systems containing Type B and C oncornaviruses to determine the site of action utilizing image intensification.

Major Findings:

Critical point dried oncornaviruses have been identified by Ferritin labeled antibodies, most notably Rauscher leukemia and mouse mammary tumor virus. The method is rapid, reliable, and easy to perform in contrast to thin sections of embedded virus pellets. The method not only allows for the detection of virus but characterizes it in the electron microscope. When used in conjunction with radioimmunoassay, it should prove a most valuable tool in the rapid identification of animal viruses.

As a means of evaluating the efficiency of the image intensification system, a heavy metal, 5-tungsto-2 antimoniate (TA) was incorporated into both B and C type oncornavirus producing cell lines. Because this compound was reported to be an RDDP inhibitor, no virus production was expected and therefore, the site of this inhibition could be visualized. The compound (TA) was found to produce increased titers of virus when combined with dexamethasone (DXM). Virions, both B and C released after treatment are morphologically complete, yet there is a significant decrease in RDDP activity. The compound was found to localize in the region of the ribosomes. The infectivity of these virions and their antigenic qualities are being investigated.

Normal human fibroblasts have been differentiated from tumor cells of human origin in tissue culture by SEM. Normal cells at late exponential growth are flat, parallel in growth alignment and void of surface modifications. In contrast, tumor cells in vitro have very modified active surfaces being covered with microvilli, ruffles, blebs and filopodia. Normal cells and cells from tumors of epithelial origin, carcinomas and melanomas, are closely associated with each other whereas other human tumor cells, rhabdomyosarcomas and glioblastomas, are quite independent of one another in vitro. The visualization of these cells in the SEM was accomplished by a new method of evaporating conductive coatings, sputtering, onto the surface of biological specimens that avoids damaging high temperatures. These studies will serve as an extremely useful method of determining malignant versus normal cells.

Significance to Biomedical Research and the Program of the Institute:

Immunochemical tagging should become an excellent tool to probe candidate human cells for the presence of subviral or intact viral information. Image intensification will enable the investigator to localize the specific site of

action of heavy atoms at the sub-cellular level which should indicate the site of viral organization and production in cells. The application of scanning electron microscopy to Viral Oncology is proving to be a very useful analytical and quantitative tool which saves a great number of man hours and cost especially in the quality control areas.

Proposed Course:

Major emphasis will be placed on the further development of energy dispersive X-ray identification of subviral entities in human cell lines and additional effort will be afforded the studies involving the use of RDDP inhibited virus production and its subsequent infectivity.

Honors and Awards:

Participant in Franco-American Exchange Program at:
Laboratory of Experimental Medicine
College de France
Paris, France
April, 1974 - July, 1974

Publications:

Aaslestad, H.G., Twardzik, D.R., Armstrong, G.R., Zeve, V.H., and Ablashi, D.V.: Partial purification and biochemical characterization of Herpesvirus saimiri (HVS). Second International Symposium on Oncogenesis and Herpes Viruses, Nuremberg, Germany. In Press. 1974.

Zeve, V.H., Gonda, M.A., Lebiedzik, J.: Application of an automated particle system to the quantitation of virus particles. J. Nat. Cancer Inst. 53: 1099-1102, 1974.

Serial No. Z01-CP-04802-02-ADVO

1. Office of the Associate Director, Viral Oncology
2. Division of Cancer Cause and Prevention
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Molecular Studies of Human and Animal Cancer

Previous Serial Number: Same

Principal Investigator: Dr. Jeffrey Schlom

Other Investigators: None

Cooperating Units: Inside NIH
Dr. J. Dahlberg, OAD, NCI

Outside NIH
Dr. J. Verna, Meloy Laboratories, Springfield, Va.
Dr. D. Colcher, Meloy Laboratories, Springfield, Va.
Dr. J. Schochetman, Meloy Laboratories, Springfield, Va.
Dr. R. Michalides, Meloy Laboratories, Springfield, Va.
Dr. P. Kimball, Meloy Laboratories, Springfield, Va.
Dr. M. Boehm-Truitt, Meloy Laboratories, Springfield, Va.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

These studies are aimed at the use of techniques in molecular biology to determine a viral involvement in animal and human mammary carcinoma. The program is divided into several major areas of interest: (a) molecular studies of the mouse mammary tumor viruses (MMTVs), (b) molecular studies of Mason-Pfizer Monkey Virus (MPMV) and related isolates, and (c) biochemical evidence for an oncornavirus in human milk and human breast tumors.

Major Findings: Radioactively labeled 60-70S RNA of various MMTVs (GR, RIII, Balb/cfC3H) were obtained from cell cultures of mammary tumors and shown to be free of contaminating murine leukemia viruses by a variety of techniques. These RNA's were used in competition hybridization experiments to determine the relationship of the genomes of various MMTVs to one another. Direct molecular hybridization experiments of these ^{3}H 60-70S RNAs, to the DNAs of various organs of several mouse strains, was used to determine the copy number and distribution of MMTV proviral DNA. The proteins of MMTV from various mouse strains have also been compared.

The protein constituents of MPMV were also studied. MPMV was grown in four different cell lines (CMMT, Rhesus foreskin, AV3 and Human NC-37) and the proteins of purified virus analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). The glycoproteins of MPMV, grown in different cell lines, were also analyzed via labeling of cultures with radioactive glucosamine and compared to those of MMTV and MuLV.

The size and subunit structure of the RNA of Mason-Pfizer Monkey Virus (MPMV) was studied and compared to Rauscher murine leukemia virus (MuLV) RNA. Dissociation of 60-70S RNA by heat or formamide resulted in conversion to identical subunit structures of 2.8×10^{6} daltons for both MPMV and MuLV RNA. Treatment with lower amounts of formamide revealed a partial dissociation to intermediate RNA structures. Complete heat dissociation of MPMV 65S RNA also released three low molecular weight RNA species.

Radioactive DNA (^{3}H-cDNA) complementary to the RNA of Mason-Pfizer Monkey Virus was used in molecular hybridization experiments to demonstrate sequence homology between MPMV RNA and RNA of human malignant breast tumors. Hybridization products were analyzed by cesium sulfate equilibrium density centrifugation and hydroxylapatite chromatography. Twenty-seven of thirty-nine human malignant breast tumors tested contained MPMV-related RNA while no sequence homology was found in RNA from human benign breast tumors or normal breast, spleen, liver or kidney. Hybridizations between MPMV ^{3}H-cDNA and human breast adenocarcinoma RNA showed a $C_{r}t_{1/2}$ of approximately 2×10^{4}. Thermal analysis of the hybrids demonstrated a T_{m} of approximately 80°C. Hybridization of radioactively labeled MPMV 60-70S RNA of breast tumor DNA is in progress.

Particulates with the properties of cores and/or ribonucleoproteins of RNA tumor viruses have been isolated from Sterox-SL-treated fractions of murine and human mammary adenocarcinomas. These particulates have an RNA-directed DNA polymerase, a 60-70S RNA, and a density of 1.26 g/ml or greater in sucrose equilibrium density gradients. Their uniquely higher densities lead to banding in regions comparatively free of cellular contaminants. These circumstances minimize some of the technical complications of performing the simultaneous detection assay in the presence of cell debris. Further purification and characterization of the reverse transcriptase from human malignant breast tumors, human milk, and human nipple discharge is now in progress.

Significance to Biomedical Research and the Program of the Institute:

These studies are aimed at the isolation and characterization of a putative breast cancer virus from human milk and malignant breast tumors.

Proposed Course:

To continue the studies described especially as they concern the biochemical and immunological characterization of virus-like particles derived from human milk and breast tumor and from cultures infected or co-cultivated with these materials. These studies will be supported for further basic experiments using the mammary tumor virus model as well as other animal model systems.

Honors and Awards:

None

Publications:

Michalides, R., Feldman, S., Spiegelman, S., and Schlom, J. Comparative studies on the molecular basis for a viral involvement in murine and human mammary adenocarcinoma. Tumor Host Virus Interaction, NATO Advanced Study Institute, 1973 (in press).

Schlom, J., Michalides, R., Colcher, D., Feldman, S., Spiegelman, S. Evidence for an RNA tumor virus in human milk. Comparative Leukemia Research, 1973, Y. Ito and R.M. Dutcher, ed., Univ. of Tokyo Press, Tokyo, pp 419-430, 1974.

Perk, K., Michalides, R., Spiegelman, S., Schlom, J. Biochemical and morphological evidence for presence of an RNA tumor virus in pulmonary carcinoma of sheep (Jaagsiekte). J. Nat. Cancer Inst. 53: 131-135, 1974.

Spiegelman, S., Schlom, J., Axel, R., Hehlmann, R., and Kufe, D. "Molecular Probing for a Viral Etiology of Human Cancer." In: Molecular Studies in Viral Neoplasia, The Williams & Wilkins Co., pp 547-573, 1974.

Yaniv, A., Ohno, T., Kacian, D., Colcher, D., Witkin, S., Schlom, J., and Spiegelman, S. Seriological analysis of reverse transcriptase of the Mason-Pfizer Monkey Virus. Virology 59: 335-338, 1974.

Spiegelman, S., Baxt, W., Kufe, D., Peters, W.P. and Schlom, J. Sequences related to the RNA tumor viruses in the RNA and DNA of human leukemias and lymphomas. Comparative Leukemia Research, 1973, Y. Ito and R.M. Dutcher, ed., Univ. of Tokyo Press, Tokyo, pp 1-25, 1974.

Schlom, J. and Spiegelman, S. Breast cancer: molecular basis for a viral etiology. New York State J. of Med., pp 1373-1384, 1974.

Colcher, D., Spiegelman, S., and Schlom, J. Sequence homology between the RNA of Mason-Pfizer Monkey Virus and the RNA of human malignant breast tumors. Proc. Nat. Acad. Sci. 71: 4975-4979, 1974.

Spiegelman, S., Axel, R., Baxt, W., Kufe, D., and Schlom, J. Human cancer and animal viral oncology. Cancer 34: 1406-1420, 1974.

Schlom, J., Colcher, D., Michalides, R., Schochetman, G., Young, J., Spiegelman, S., and Feldman, S. Biochemical evidence for a viral involvement in human breast cancer. Fundamental Aspects of Neoplasia, A.A. Gottlieb, ed., Springer-Verlag, New York (in press).

Michalides, R., Spiegelman, S. and Schlom, J. Biochemical characterization of putative subviral particulates from human malignant breast tumors. Cancer Research (in press).

Schlom, J. Biochemical evidence for a viral involvement in human breast cancer. Report to the Profession, Breast Cancer Task Force, pp 55-61, 1974.

Colcher, D., Spiegelman, S., and Schlom, J. Sequence homology between MPMV cDNA and the RNA of human malignant breast tumors. Ninth Meeting on Mammary Cancer in Experimental Animals and Man, pp 79, 1974.

Michalides, R., Kimball, P. and Schlom, J. Detection of MMTV sequences in murine DNA: A method to determine the interrelationship between the various MMTV strains. Ninth Meeting on Mammary Cancer in Experimental Animals and Man, pp 55, 1974.

Schlom, J., Colcher, D., Schochetman, G., Michalides, R., Boehm-Truitt, M., and Kimball, P. Biochemical studies of Mason-Pfizer Monkey Virus. Ninth Meeting on Mammary Cancer in Experimental Animals and Man, pp 80, 1974.

Schochetman, G., Kimball, P., and Schlom, J. RNA subunit and protein structure of Mason-Pfizer Monkey Virus. Ninth Meeting on Mammary Cancer in Experimental Animals and Man, pp 78, 1974.

Schlom, J., Colcher, D., Schochetman, G., Michalides, R., Boehm-Truitt, M., and Kimball, P. Biochemical studies of Mason-Pfizer Monkey Virus. Symposium on the Characterization of Oncorna and Related Viruses, pp 46, 1974.

Schochetman, G. and Schlom, J. RNA subunit structure of Mason-Pfizer Monkey Virus. J. Virology 15: 423-427, 1975.

Colcher, D., Spiegelman, S., and Schlom, J. Relationship of Mason-Pfizer virus to human breast cancer. Annual Meeting of Breast Cancer Task Force, pp 49, 1975

Colcher, D., Schochetman, G., and Schlom, J. Mason-Pfizer Monkey Virus (MPMV): A horizontally transmitted oncornavirus of rhesus monkeys. American Association for Cancer Research Symposium.

Aaronson, S.A. and Schlom, J. The search for RNA tumor viruses in human cancer. Progress In Clinical Cancer, Irving M. Ariel, ed., Grune & Stratton, Inc., New York (in press).

Spiegelman, S., Axel, R., Baxt, W., Kufe, D., and Schlom, J. The molecular genetics of human cancer and its etiological implications. Genetics (in press).

Serial No. Z01-CP-04803-03-ADVO
1. Office of the Associate Director, Viral Oncology
2. Division of Cancer Cause and Prevention
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Ultrastructure and Morphogenesis of A, B and C Type Virus Particles
B. Histopathological Response of Tumor-Bearing Animals to Chemo- and Immunochemotherapy.

Previous Serial Number: Same

Principal Investigator: Dr. Kalman Perk

Other Investigators: None

Cooperating Units: Inside NIH

Dr. M. Chirigos
Dr. A.J. Dalton
Dr. J. Dahlberg
Dr. J. Pearson
Dr. J. Schlom

Outside NIH

Flow Laboratories, Inc., Rockville, Maryland
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

A. To analyze the morphogenesis and structure of the virus-like particles associated with the L2C transplantable leukemia of the guinea pig and to compare these virus particles to the type B virus induced in tissue cultures of guinea pig cells by 5-bromo-2'deoxyuridine. This comparison was both morphological and biochemical.

B. To examine the histopathological host response resulting from a variety of therapeutic protocols as applied to tumor systems of the mouse, rat and guinea pig.

Methods Employed:

Histochemical, immunological, biochemical, tissue culture and electronmicroscopic methods were employed.

Major Findings:

A. The virus released from guinea pig tissue culture cells following treatment with BrdU was shown to have many of the morphological features of the murine type B virus, MMTV. In an effort to substantiate this report of a new type B virus, biochemical tests were developed to compare the BrdU-induced guinea pig virus with murine type B and type C viruses.

Equilibrium centrifugation of the guinea pig virus in sucrose and cesium chloride indicated that its buoyant density is 1.19 g/cm^3 and 1.21 g/cm^3 respectively, identical to the values for MMTV, while murine type C virus has a density of 1.16 g/cm^3 in both solutes.

The reverse transcriptase of the guinea pig virus was then tested for its divalent cation preference. It was found that Mg^{+2} was preferred, with 20mM being the optimal concentration. Mn^{+2} was completely ineffective at all concentrations tested. Again, these reaction conditions are identical to those preferred by MMTV but strikingly different from those preferred by mammalian type C viruses.

In preliminary attempts to test the guinea pig virus against antisera to MMTV and type C viruses, no antigenic relationship has been found to any other virus. Attempts to hybridize the BrdU-inducible guinea pig virus to the DNA product of other viruses have also been uniformly unsuccessful.

It therefore appears that although the murine type B virus, MMTV, and the inducible guinea pig virus share many morphological and biochemical properties, they are not closely related in an evolutionary sense.

Further efforts were directed towards a comparison of the inducible guinea pig virus with the virus-like particles present in the plasma of strain 2 guinea pigs inoculated with the transplantable L2C leukemia. These particles have been well described in the literature and there seems to be little doubt that they are derived from the intracisternal particles present in tumor cells by a process that does not involve budding from the plasma membrane since this has never been observed. Preliminary results indicate that a reverse transcriptase activity, apparently identical to the enzyme present in the inducible tissue culture-grown virus, is present in the plasma of leukemic guinea pigs. Since the morphological evidence indicates that virus resembling the BrdU-inducible particles is not present in tumor cells of the leukemic

guinea pigs, our current interpretation of the biochemical data is that during the late stage of the leukemia, some event occurs which accidentally or otherwise triggers the production of the type B virus, presumably in normal cells, since it is not observed in the tumor cells. Current efforts are being directed towards explaining the mechanism of production of this virus and attempting to determine where the virus originates.

B. Histopathological examinations were performed on murine, rat and guinea pig tumor systems following a variety of chemical and immunochemical therapies. Of particular interest was the finding that methyl CCNU (1-(2-chloroethyl)-3-(trans-4-methylcydohexyl)-1-nitrosourea) could prevent meningeal leukemia development in guinea pigs receiving L2C tumor cells and, as a result, led to permanent remission (up to 176 days). This may indicate the potential use of methyl CCNU in the treatment and/or prevention of meningeal leukemia development in human leukemia.

Significance to Biomedical Research and the Program of the Institute:

A. Of primary significance is the finding that type B viruses are not restricted to mice. In addition, a variety of biochemical and biophysical methods are proving useful in extending morphological distinctions between B and C type viruses.

The discovery of a reverse transcriptase activity in the plasma of leukemic guinea pigs is of considerable interest because the morphological findings failed to demonstrate any of the type B virus in the tumor cells. It appears unlikely that the intracisternal virus present in the tumor cells would have an enzyme identical to that present in an entirely different virus, and clarification of the mechanism of how the enzyme appears in the plasma of these guinea pigs may shed illumination on the mechanism of induction of many different viruses.

B. The better understanding of the host response following drug therapy alone or in combination with immunostimulators could lead to more effective therapies in human disease. The discovery that methyl CCNU prevented meningeal leukemia in the guinea pig could possibly aid in the treatment of leukemic children.

Proposed Course:

A direct role in this program was terminated in November, 1974, due to my return to a permanent position at the Hebrew University in Israel.

Honors and Awards:

None

Publications:

Dahlberg, J.E., Perk, K., Dalton, A.J.: Virus-like particles induced in guinea pig cells by 5-bromo-2'deoxyuridine are morphologically similar to murine B type virus. Nature 249: 828-830, 1974.

Perk, K., and Dahlberg, J.E.: Murine intracisternal A type particles fail to separate from the membrane of the endoplasmic reticulum. J. Virol. 14: 1304-1306, 1974.

Perk, K., Pearson, J.W., Torgersen, J.A. and Chirigos, M.A.: An animal model for meningeal leukemia. Int. J. Cancer 13: 863-866, 1974.

Perk, K., Chirigos, M.A., Fuhrman, F., and Pettigrew, H.: Some aspects of host response to Levamisole after chemotherapy in a murine leukemia. J. Natl. Cancer Inst. 54: 253-256, 1975.

Pearson, J.W., Perk, K., Torgersen, J.A. and Chirigos, M.A.: Drug therapy against a transplantable guinea pig leukemia. J. Natl. Cancer Inst., in press.

Pearson, J.W., Perk, K., Chirigos, M.A., Pryor, J.W., and Fuhrman, F.S.: Histological and combined chemoimmunostimulation therapy studies against a murine leukemia. Int. J. Cancer, in press.

Serial No. Z01-CP-04990-01-ADVO
1. Office of the Associate Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Regulation and expression of mammalian RNA tumor viruses.

Previous Serial Number: None

Principal Investigator: Dr. Robert J. Goldberg

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. Wade Parks, VCB, VO, DCCP, NCI
Dr. Edward Scolnick, VLLB, VO, DCCP, NCI

Outside NIH:

Dr. Frank J. O'Neill, University of Utah, Salt Lake City, Utah

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

(1) To characterize biochemically and biologically cell transformation by RNA-containing tumor viruses and to study the factors regulating the expression of RNA tumor viruses in vitro. (2) To isolate and characterize new RNA tumor viruses.

Methods Employed:

Various tissue culture assays and procedures have been employed including: cloning of cells in soft agar and microtest II plates, focus-assays for type C helper viruses, somatic cell hybridization, co-cultivation procedures, infectious center assays, establishment of primary cell cultures from a variety of animal tissues, and the induction and propagation of a variety of RNA tumor viruses in cultured cells.

Biochemical methods used during the last year have included isolation and purification of viral and cellular nucleic acids, the use of s1 nuclease for hybridization assays, the synthesis of DNA transcripts by use of the endogenous reaction of RNA tumor viruses, subcellular fractionation procedures, and the separation and characterization of various classes of RNA.

Major Findings:

Several approaches have been employed to study the factors which regulate the expression of endogenous and exogenous RNA tumor virus genetic information. One approach has focused on the selection of somatic mutants of various mammalian cells following exposure to potent mutagens under carefully controlled conditions. Mutagenized cells were plated under conditions designed to select variants with known genetic markers. The cells were cloned and continuously propagated under selective conditions to prevent the appearance of wild-type revertants in the population. In some cases, somatic cell hybrids were selected following fusion of appropriate mutant cells with inactivated Sendai virus. In other cases, the virologic characteristics of the mutant cells and their wild-type counterparts were studied directly. Some of the parameters chosen for characterization have included the inducibility of these cells for type B and type C virus expression and their susceptibility to exogenous infection with different type C viruses.

The biology of rat type C viruses has been studied during the past year. Cell cultures derived from 10 inbred strains of rats were characterized with regard to the inducibility and host range of their endogenous type C viruses. Using an extremely sensitive plating technique, a type C virus obtained from a cell culture derived from an Osborne-Mendel rat has been successfully transmitted to cells of a heterologous species. The fidelity of the transmission has been confirmed by immunological, biochemical, karyological and biological tests. Attempts are presently underway to transmit other rat type C viruses to heterologous cells and to study the biochemical and immunological properties of these viruses.

Work performed in this laboratory over the past three years has shown that the Kirsten and Harvey murine sarcoma viruses are genetic recombinants between a mouse type C virus and genetic information of rat origin. During the last six months, studies have been conducted to more fully characterize the nature of the RNA genetic information contained in these murine sarcoma viruses. Specific probes for the different sequences of the sarcoma virus genome have been used to study their distribution in various normal and malignant rat tissues. The physical properties and translatorial programs of these sequences are currently being investigated.

Investigations of several human cell lines and viruses have been conducted as part of a joint agreement between cancer virologists in the U.S.A. and the U.S.S.R. As part of these studies, a type C RNA virus has been isolated from various tissues of a lymphomatous baboon (sp. P. Hamadryas). Virus

isolations were made by co-cultivating baboon cells from the inguinal and mesenteric lymph nodes, testes, kidneys and spleen with cells of canine or human origin. The isolated virus grew in canine, bat, rhesus and human cells but not in cells of mouse, rat, cat or rabbit origin. The baboon isolate resembled a type C virus when infected cells were examined by thin section in the electron microscope. In addition, the virus was capable of providing helper function by rescuing and transmitting the Moloney and Kirsten sarcoma virus genome from non-productively transformed cells. Antibody directed against the RD114 virus reverse transcriptase was very effective in inhibiting the baboon virus polymerase while anti-mouse and woolly type C virus polymerase antibodies had no significant inhibitory activity. Further analysis by immunodiffusion and competitive radioimmune assay revealed a close immunological relationship between this virus, RD114 and another type C virus isolated from the placenta of a different species of baboon. Finally, three different classes of interspecies antigenic determinants have been demonstrated in mammalian type C viruses.

Significance to Biomedical Research and the Program of the Institute:

Oncornaviruses have now been isolated from a variety of mammalian and avian species. Many of these viruses have been shown to be involved etiologically in the induction of malignant diseases. Although type C or type B viruses have not been isolated from human cells, sub-viral footprints have been detected in various human tissues. In addition, type C viruses from several mammalian species have been shown to replicate in, and in some cases, transform, human cells. Therefore, it is obvious that these self-contained packages of genes offer a convenient and relevant means to study the mechanism of cell transformation. It is also imperative that the search for a bona fide human tumor virus be pressed. Ultimately this would facilitate a thorough study of the involvement of this class of viruses in human neoplasia and open a rational approach for immunoprophylaxis and therapy of these diseases.

Proposed Course:

Efforts during the next year will be directed into two areas. First, the transcriptional and translational events surrounding cell transformation by mammalian sarcoma viruses will be studied. Although these studies will basically revolve around a biochemical-biological approach, it is anticipated that later phases of the project will include some immunochemical studies. Secondly, attempts will continue to isolate RNA tumor viruses and to detect virus information in various mammalian species, especially from higher primates including man.

Honors and Awards:

None

Publications:

Goldberg, R.J., Scolnick, E.M., Parks, W.P., Yakovleva, L.A. and Lapin, B.A.: Isolation of a primate type C virus from a lymphomatous baboon. Int. J. Cancer 14: 722-730, 1974.

Scolnick, E.M, Goldberg, R.J., and Parks, W.P.: A biochemical and genetic analysis of mammalian RNA-containing sarcoma viruses. Cold Spring Harbor Tumor Virus Symposium 39, 1974, in press.

d. Scientific Information Management Unit

The Information Unit within the Office of the Associate Director is responsible for the collection and dissemination of scientific information in the area of virus cancer research. Services are available to all staff members, VCP contractors and, as far as possible, to persons outside the Viral Oncology Area. Major activities during FY75 have been:

1. Bibliographic Service: A semi-automated system is maintained for storage, rapid search, identification, and reference print-outs covering topics in the published literature on viral oncology. The system has recently been updated to include 1600 additional articles. The latest citations in virology literature are available.

2. Cooperative Literature Searching Service: Cooperative arrangements from this office have been established with the Information Offices of six other federal agencies whereby their literature collections and other user services are made available to Viral Oncology program staff members on request.

3. Contractors' Progress Reports: Annual and semi-annual progress reports are requested on schedule from Virus Cancer Program contractors and distributed to program management personnel and review committees. Condensed summaries of these reports are compiled and distributed to program scientists.

4. Library: A small library facility is maintained in Building 37 for the use of Viral Oncology staff. Over forty scientific journals are received regularly and over three hundred volumes, monographs, and other publications are collected.

5. Viral Oncology Contractor Directory: This publication contains the names, addresses, and telephone numbers of contractors, principal investigators, project officers, and contract specialists within the Viral Oncology Program. Its purpose is to provide up-to-date information to facilitate and expedite communications between staff members and contractors.

6. VCP Bibliography: Citations to all papers published by Viral Oncology staff members and research contractors are available upon request. Special listings are prepared for the NCI annual report and the VCP annual progress report.

7. Program Monitoring System: A management information system for Viral Oncology is being implemented to provide budgetary, scientific and administrative information. It will be the responsibility of this office to maintain the system, updating data directly on an IBM terminal using WYLBUR, a text-editor and remote job entry service. Information required for a great variety of purposes will be instantly retrievable.

3. Branch Reports a. VIRAL BIOLOGY BRANCH
July 1, 1974 - June 30, 1975

The Viral Biology Branch conducts research on viruses and host factors related to carcinogenesis and on the development and evaluation of measures for cancer control in experimental systems. Investigations are conducted to detect and identify the nature of virus activity expressed in tumor tissue, study the effect on tumorigenesis of interaction between viruses co-infecting the host cell, determine the biological behavior of neoplastic cells, characterize virus and tumor-specific antigens, and examine biochemical events related to viral infection and cell transformation. Selected experimental cell culture and animal systems are used to evaluate viral relationships to the carcinogenic process and the effectiveness of combined chemotherapeutic and immunotherapeutic measures in the control of virus activation and tumor growth.

The Cell Biology Section investigates the behavior of malignant cells cultured in vitro. The correlates of malignancy are identified for use in the genetic analysis of the malignant state by cell fusion techniques. The immune responses of the host to cell surface neoantigens associated with cellular transformations to malignancy are studied as a means for the development of methods for immunodiagnosis and immunotherapy applicable to humans.

The Microbiology Section has emphasized investigations on herpesvirus-induced malignancies, particularly in nonhuman primates. Attention is directed to the immune responses of the host after virus infection and the correlation of functional immunity with the course of neoplastic disease in the animal.

The Human Tumor Studies Section combines virological, immunological and biochemical techniques to study virus-virus interactions in relation to neoplasia, to characterize the protein products of virus gene expression, and to determine the nature of the association established between the virus genome and the cell in chronic infections.

The Virus and Disease Modification Section is concerned with the development of effective measures for the control of virus-induced neoplasms. Combined chemotherapeutic and immunotherapeutic treatments are evaluated in terms of prolonged life and the suppression or elimination of tumor growth in animals. Biochemical investigations are conducted to acquire information concerning probable mechanisms involved in effective treatments.

The Office of the Chief coordinates the research activities of the Sections within the Branch with due recognition to the scientific freedom of individual investigators engaged in fundamental studies. The Office is responsible for establishing collaborative efforts between investigators outside, as well as within the Branch. The Chief serves as the Chairman of the Developmental Research Segment within the Virus Cancer Program and is assisted in this capacity by the Vice-Chairman and the Executive Secretary for the Segment. As such, the Chief is responsible for the maintenance of a collaborative contract research effort contributing to the overall effort in the Viral Oncology Area.

Investigators in the Cell Biology Section, who have studied virus infection of cultured tumor cells as a means to increase the antigenicity of weak tumor cell antigens, have now prepared antigen fractions from cultured, influenza virus-infected, human cancer cells. These preparations are awaiting formal approval for trials as immunodiagnostic reagents. In some instances, such substances may have value in immunotherapy applied to specific types of cancer.

In studies of cell-mediated immune responses to specific tumor cells in mice, the delayed type hypersensitivity reaction becomes suppressed as the tumor load increases. Under conditions optimum for demonstration of cell-mediated sensitivity to specific tumor cells, immunological assay detected cross-reactivity in 3 tumor cell lines previously thought to be unrelated. These include SV40 transformed BALB/c mouse 3T3 cells, herpes simplex type 2 virus-transformed embryonic mouse skin cells, and LS9-3, a mouse cell line, originated from a methylcholanthrene-induced squamous cell carcinoma. Similar cross-reactivity was not observed with other tumor lines tested or with syngeneic embryonic or adult mouse fibroblasts.

Mouse tumor cells extracted with 3M KCl yield soluble antigens which react positively in delayed hypersensitivity tests but do not protect mice in the tumor rejection assay. The extraction of the tumor antigens from the cells is not complete; following the extraction procedure, the cells possess full tumor rejection activity, as well as strong activity when used in delayed hypersensitivity tests. One may hypothesize that the extraction procedure partially removes an incomplete tumor transplantation antigen and, at the same time, completely removes inhibitory or blocking factors which usually make untreated killed tumor cells poor immunogens.

The kinetics of feline leukemia biosynthesis was studied in the presence of different inhibitors. At least 2 RNA species with different life times and processing requirements participate in virion assembly. The shortest lived mRNA required for virus production turns over in 2 to 4 hours, while the rate limiting mRNA necessary for cell surface antigen synthesis persists for 10 hours. At least half of feline leukemia virus infected cell surface antigens was identified as the 27,000 dalton virus structural protein.

Eight isozyme systems were examined in 20 methylcholanthrene-transformed cell lines, and considerable quantitative as well as qualitative variation was found in a number of these lines. The isozyme aberrations are being used as cell markers in cell fusion experiments designed to examine the genetic regulation of gene dysfunction and malignancy following neoplastic transformation.

The functional immune status of animals during the course of virus-induced neoplastic disease has been under study in the Microbiology Section. Sera from mice in which tumors induced by the Moloney sarcoma virus-leukemia virus complex have regressed are found to contain high titers of antibodies reacting with virus-induced cell membrane antigens as determined by immunofluorescence methods. The effectiveness of these sera in tumor immunity was evaluated by passive transfer followed by challenge with two tumor cell types: virus-producing sarcoma and leukemia genome-positive (S+L+) mouse cells and

non-producing sarcoma genome-positive, leukemia genome-negative (S+L-) mouse cells. The sera were protective only against virus-producing cells, indicating that any antigenic stimulus resulting from the sarcoma moiety of the sarcoma-leukemia virus complex was insufficient to influence significantly the production of antibodies directed against any sarcoma-specific antigens that might be expressed at the cell membrane.

A number of attempts were made to detect a sarcoma-specific antigen on the membranes of non-producer S+L- cells. Suggestive evidence for the presence of a weak antigen of this type has been observed. Sera from mice with progressively growing tumors induced by the Moloney sarcoma-leukemia virus complex contain factors enhancing growth of these virus-caused tumors. Such sera enhanced the growth of S+L- cell tumors but had no effect on the uninfected parental cells. Further, spleen cells removed from mice between 2 and 3 weeks after infection by virus of the sarcoma-leukemia complex specifically killed S+L- cells.

Antibody-dependent lymphocyte cytotoxicity also may have some activity in immunity directed against tumors induced by the Moloney sarcoma-leukemia virus complex. The sera from mice in which tumors had regressed actively mediated the destruction of Moloney virus-producing cells by normal or immune lymphocytes. This activity was detected only in the sera from "regressor" mice.

In other laboratories, a relationship had been shown to exist between the persistence of antibodies to "early antigens" induced in cells by Epstein-Barr virus and the prognosis for Burkitt's lymphoma patients in therapeutically-induced remission. An analogous relationship between antibody to the "early antigens" induced by Herpesvirus saimiri and the disease process in infected animals has been under study. The temporal relationship previously observed between the development of lymphoma and the rise in antibodies to these "early antigens" in the infected owl monkey has been confirmed. The titer of these antibodies is quantitatively related to the number of peripheral blood lymphocytes which carry recoverable virus. Thus, in the capuchin monkey which carries the virus genome in low to moderate quantity in peripheral blood lymphocytes in the absence of disease, low levels of antibody to the "early antigens" induced by Herpesvirus saimiri are maintained. It is interesting that sera with high activity in tests for antibody-dependent lymphocyte cytotoxicity for owl monkey kidney cells infected by this virus comes from animals with the malignant disease.

The DNA extracted from Herpesvirus saimiri was characterized within the Human Tumor Studies Section and found to contain two DNA species with densities of 1.727 gm/cc and 1.709 gm/cc in cesium chloride density gradients. When the virus was treated with DNase prior to DNA extraction, four DNA species were obtained with densities of 1.727, 1.718, 1.712 and 1.706 gm/cc. The relationships between these nucleic acid species will be investigated.

The results of dual herpesvirus infection were determined in the P3HR1 lymphoblastoid cell line. This culture carries Epstein-Barr virus (EBV), and about 10% of the cells contain demonstrable EB virus capsid antigens at any one time. Superinfection of these cells with herpes simplex virus type 1

(HSV-1) did not result in cell lysis. Instead, a chronic HSV-1 infection was established. While only a few cells produced HSV as estimated by infectious center assays, HSV antigens could be demonstrated in 80 percent of the cells. Apparently, the replication of both EBV and HSV is regulated by the host cell and this system offers the opportunity for investigation of the mechanism for control. Further study of this system is planned to determine whether the DNA of these viruses is integrated within the DNA of the cell, whether the DNA packaged in virions is a composite of the DNA of both viruses, and whether HSV production is cell cycle dependent.

Dual infection of cells with herpes simplex virus and an RNA type-C virus was also examined. When mouse cell cultures (JLSV-9) chronically infected with Rauscher murine leukemia virus were superinfected with HSV-1, the HSV progeny produced syncytial lesions on chicken embryo fibroblasts in contrast to the round cell plaques produced by the parental virus stock. The HSV variant is lighter in cesium chloride gradients than the parental stock, preliminary hybridization shows some differences from the DNA of the parent virus, and restriction enzyme DNA fragments also show differences between the two DNAs. Of further interest is the ability to isolate viable Vero cell clones after infection of the cells by the syncytium-producing virus whereas this has not been possible with the HSV-1 parental stock. These clones offer an opportunity to determine which HSV DNA fragments may persist within the cells and may provide a DNA probe for the examination of malignant human cells for the presence of HSV genetic information.

A large 60,000 molecular weight polypeptide, p60, has been identified in the feline leukemia virus (FeLV) pseudotype of Moloney sarcoma virus (MSV). In this virus complex, this polypeptide exists in 2- to 3-fold excess over the content of the MSV p30 peptide and in 8-fold excess over the FeLV p27 peptide. Characterization of the p60 component suggests that it is a precursor to murine virus p30. The observation supports the probability that the RNA tumor virus messenger RNA is translated as polycistronic protein products which are subsequently cleared into functional polypeptides. This raises the possibility that MSV-induced cell transformation may be related to aberrant protein cleavage due to a reiterated or faulty recombined message.

Five chemicals and 3 biologicals capable of stimulating host immune responsiveness have been tested in mice in the Virus and Disease Modification Section for use as immunoadjuvants against several leukemia and carcinoma tumor systems in combined modality therapy studies. Of the 5 chemicals, levamisole has been examined in depth. The activity of this chemical immunoadjuvant is expressed by stimulating the T-lymphocyte population. Results of *in vitro* and *in vivo* testing with levamisole indicate this chemical is capable of inducing blastogenic stimulation of spleen lymphocytes, enhancing allogeneic stimulation in mixed lymphocyte cultures (MLC), and stimulating DNA synthesis of resting lymphocytes, but not fibroblasts, macrophages or tumor cells. This stimulation appears to be mediated by the activity of levamisole on the cyclic GMP-cyclic AMP "second messenger" complex via interaction with phosphodiesterase. The effectiveness of levamisole as an immunoadjuvant in chemotherapeutically-induced remissions of leukemias and carcinomas has led to its incorporation into clinical protocols designed to more effectively treat human leukemia and colorectal adenocarcinomas. Studies are continuing with 3 biological agents

which possess immunoadjuvant activity: Staphylococcus aureus, C. parvum, and interferon. Studies in model animal tumor systems indicate that immunoadjuvant therapy is consistently effective when applied in a host with a reduced tumor burden achieved by chemotherapy and/or surgery.

Results, attained with combined chemotherapy of the Gross leukemia guinea pig model, show the importance of employing a drug possessing both antitumor activity and lipid solubility. Of the many drugs tested, only Methyl CCNU was capable of controlling systemic leukemia and preventing the development of meningeal leukemia. These results were attained due to the ability of the drug to penetrate the hosts' blood-brain barrier.

Homogenous cell lines of human colon and rectal adenocarcinoma (Ca) have been established. A significant number of sera from patients with colon or rectal Ca demonstrated cytotoxicity against these cell lines. This cytotoxicity is complement-dependent and appears to be specific for colonic and/or rectal Ca cells. Similarly, peripheral blood lymphocytes from colorectal Ca patients and patients with other entodermally-derived malignancies inhibit colony formation of these established cell lines. Of particular interest is that abrogating factors (blocking antibody) of this cell-mediated cytotoxicity could be demonstrated in serum of patients with advanced Ca of the colon, esophagus, liver, rectum, and stomach, but not with non-entodermal cancers. These cell lines are presently employed for the purpose of providing meaningful information on the hosts' immune response to colorectal neoplasms.

A potent inhibitor of avian myeloblastosis virus (AMV) and Rauscher leukemia virus (RLV) derived RNA-dependent DNA polymerase (RDDP) has been detected in tumor cell extracts of human colorectal Ca. The extracted material does not inhibit bacterial (M. luteus) or cellular (JLSV-9) DNA polymerases. Extracts of Hela cells, A1-2 cells, or human skin fibroblasts lack this inhibitor. Partial characterization of this inhibitor has been completed.

Two lines of murine leukemia, L1210 and MBL-2, were shown to inhibit DNA synthesis of normal murine spleen lymphocytes in mixed culture. An extract of the frozen and thawed tumor cells, but not of normal lymphocytes, also possessed this inhibitory activity. Studies are underway to characterize this inhibitor.

Extracts of lymphoma cells derived from 3 different species of fish contain a viral-like RDDP activity which has a temperature optimum of 20°C and is inactive at 37°C. Only minor temperature differences in optima have been noted for RDDPs examined from murine, feline, bovine, and non-human primate sources. This is the first instance that such a major difference has been observed and may be associated with the low temperatures of the normal environment of these cold-blooded animals.

Several anthracycline compounds have been tested for their capability to inhibit RDDP of AMV and RLV. Inhibition was due primarily to the tetracyclic planar ring structure (daunomycinone) common to all of these compounds. The inhibitory activity appears to be due to their binding capacity for template and not enzyme.

Through heat inactivation studies, it was determined that RDDP showed a preference for primed templates, especially for its native 70S RNA, and that the active sites for RNase H and for RDDP activities were not identical.

Pyran copolymer, covalently linked to cyanogen bromide-activated sepharose has been shown to be an effective affinity column potentially adaptable for the general purification of viral DNA polymerase.

Other Activities of the Branch: During this reporting period, investigators in the Branch were senior authors on 18 published manuscripts, on 20 manuscripts currently in press, and collaborating authors with investigators outside the Branch on 11 published manuscripts and 11 manuscripts presently in press.

Members of the Branch presented invited lectures in this country and abroad and discussed their research findings at scientific meetings. The Branch has hosted guest workers and provided for studies by visiting scientists. Training in special procedures was provided to several individuals.

Senior members of the Branch served as Project Officers on research contracts within the Virus Cancer Program and in positions of Vice-Chairman or Executive Secretary for Segments of the Program. The Branch Chief serves as Segment Chairman for the Developmental Research Segment.

Serial No. Z01-CP-04873-01-VB
1. Viral Biology Branch
OADVO, Division of Cancer
Cause and Prevention
2. Virus and Disease Modification
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Combination Chemoimmunotherapy Using Chemical Immunoadjuvants Studied by *In Vitro* and *In Vivo* Methods

Previous Serial Number: NONE

Principal Investigator: Dr. Stephen J. Mohr

Other Investigators: Dr. Michael A. Chirigos

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other	0.0

Project Description

Objectives:

1. Establish model systems for immunotherapy of murine solid tumors and leukemias initially treated surgically or chemotherapeutically to produce remission.

2. Test chemical immunostimulators in the above systems to produce cures.

3. Determine the mechanism of action of useful immunostimulators in the above murine neoplasias by a variety of techniques.

4. Apply successful immunoadjuvant regimens to clinical use.

Methods Employed:

In vivo methods: Viral and non-viral induced murine leukemias are put into remission using a variety of chemotherapeutic regimens. Solid murine tumors are surgically excised after metastases have occurred. Immunotherapy is applied during remission using a variety of chemicals. Homografts using allogeneic tumor cells to normal mice and the effect of immunoactive drugs thereon are used to evaluate cellular immunity.

In vitro methods: Spleen weight and histology is used to evaluate immunostimulator mechanisms. Lymphocytes isolated from normal and immune stimulated mice are used in PHA leukoblastogenesis studies and release of Cr^{51} in cytotoxicity studies.

Major Findings:

Pyran copolymer, tilorone hydrochloride, levamisole, polyinosinic-polycytidylic acid and polyadenylic-polyuridylic acid appear to be capable of producing large numbers of cures in mice bearing leukemias that have been put into chemotherapeutic remission.

The survival time of mice bearing malignant melanoma metastatic disease after surgical removal of the primary tumor is improved by levamisole.

Levamisole is (1) capable of protecting mice treated with toxic doses of BCNU, (2) able to restore spleen size of drug-suppressed animals towards normal, (3) augments homograft rejection in drug suppressed and normal animals.

Pyran therapy at specific times will cause progressive tumor growth in animals bearing tumor homografts and these animals have blocking antibody as assayed by testing their serum in Cr^{51} release from cultured homograft cells by immune lymphocytes.

Significance to Biomedical Research and the Program of the Institute:

Presently, there exist three modes of therapy by which the clinician may treat his cancer-bearing patient. Surgical resection, chemotherapy, and radiation have been developed to the point that significant reductions in tumor load can be achieved. Cures are still elusive, however, since a critical small amount of residual tumor usually exists. By stimulating the immune system of the patient to seek and destroy the remaining cells, cures might be achieved. Biological immune stimulators are presently in clinical use but are hampered by pyrogenic potential (BCG) and scarcity (interferon). Chemical immunoactive agents are ideal in these respects and, therefore, deserve the greatest attention. To assess efficacy of levamisole in humans, a collaborative study with the University of Wisconsin involving chemotherapy with 5-FU and levamisole immunotherapy of post-operative patients having Duke's B or C colon adenocarcinoma has been established. The efficacy of certain chemical immunostimulators has been described herein and therefore represents a significant advance toward cancer therapy.

Proposed Course:

1. Further develop combination chemoimmunotherapeutic regimens and expand their application to other systems.

2. Further define the mechanism of action of chemical immunostimulators in terms of T-cell and B-cell function.

3. Further define the role of blocking antibody in progressive tumor growth.

4. Apply this knowledge in clinical trials involving good risk patients as soon as possible.

Honors and Awards:

None

Publications:

Mohr, S. J. and Chirigos, M. A.: Tilorone hydrochloride as immunoadjuvant therapy against murine neoplasia. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Chirigos, M. A., Mohr, S. J., and Chaparas, S. D.: Combined modality treatment with levamisole against neoplasias and protective effects. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Serial No. Z01-CP-04875-12-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Virus and Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control of Oncogenic Viruses and Induced Diseases Mediated by External Factors

Previous Serial Number: SAME

Principal Investigator: Dr. Michael A. Chirigos

Other Investigators: Dr. Wilna A. Woods
Dr. John W. Pearson
Dr. Takis S. Papas
Dr. Martin J. Fliegelman
Dr. Stephen J. Mohr

Cooperating Units: Inside NIH:
Dr. S. Chaparas, DBP, BR
Dr. V. DeVita, DCT, NCI
Dr. H. Pettigrew, B, NCI
Dr. T. Theodore, LM, NIAID
Dr. R. Young, M, NCI

Outside NIH:
Dr. M. Hirsch, Massachusetts General Hospital, Boston, Massachusetts
Dr. P. Black, Massachusetts General Hospital, Boston, Massachusetts
Dr. P. Price, Microbiological Associates, Inc., Bethesda, Maryland
Dr. I. Shif, Microbiological Associates, Inc., Bethesda, Maryland
Dr. E. Borden, University Hospitals, University of Wisconsin, Madison, Wisconsin
Dr. N. Petrakis, University of California, San Francisco, California
Dr. D. Schwartz, Children's Health Center, San Diego, California
Dr. J. G. Chirikjian, Georgetown School of Medicine, Washington, DC
Dr. D. Fish, Frederick Cancer Research Center, Frederick, Maryland

Serial No. Z01-CP-04875-12-VB

Dr. K. Perk, The Hebrew University of Jerusalem,
Rehovot, Israel

Man Years:

Total:	5.0
Professional:	1.0
Other:	4.0

Project Description

Objectives:

The overall goal of this project is to determine the most effective means of preventing and/or controlling leukemias, sarcomas, carcinomas, and melanomas. Several therapeutic modalities are examined in animal model systems to assess the most feasible approach that can be employed to exert maximum antitumor activity with minimal deleterious effect on the host. Aggressive chemotherapy often leads to reduced host immunocompetence, as well as organ and/or cell toxicity, kidney or liver damage, which are considered contributing causes of a reduced curative rate. External factors such as chemicals or biologicals which can provoke a host response to decrease the deleterious effects of chemotherapy and/or enhance immunocompetence would be beneficial when used in a combined treatment modality.

Immuno-adjuvant therapy shown to be effective in more than two animal tumor systems, and considered of potential use for supportive therapy in human cancer, will be recommended for clinical trial.

Methods Employed:

A. Virus and Tumor Animal Systems

The following are employed for *in vitro* and *in vivo* studies:

1. The Rauscher, Friend, Moloney, Graffi, and Gross leukemogenic viruses, and the Moloney, Harvey, and PV sarcomas.

2. AKR mice with spontaneous lymphoma.

3. Rat leukemias (Gross, Nova, and Dunning).

4. Lewis lung carcinoma.

5. B-16 and S-91 Melanomas.

6. DMBA and 3MC induced fibrosarcomas.

7. Rhabdomyosarcoma.

B. Humoral and cellular antibodies are measured by standard procedures or new methods developed by Section Staff.

Major Findings:

1. Immunostimulators: Several chemicals and biological materials have been tested for adjuvant activity. Five defined chemicals (Levamisole, Poly A:U, Pyran copolymer, Poly I:C, Tilorone) and *Staphylococcus aureus* extracts (membranes, cytoplasm, periplasm and mesosomes) have been found to possess activity. Levamisole has been studied in depth. When used as an adjunct to cytoreductive chemotherapy, levamisole exerted a significant protective effect when tested in two different leukemia model systems. A significantly higher number of animals survived which were leukemia free. The most effective time of administering levamisole was shown to be during the chemotherapy-induced remission period. Of particular significance were the results indicating that only one dose of levamisole was necessary to provoke this response and that this response could be obtained at doses ranging from 2 to 20 mg/kg. The levamisole effect was not dependent upon the route of administration. Based on histological data, the current interpretation of the mechanism of levamisole's activity is that it mediates a more rapid and earlier response of lymphocytic replication. Most chemotherapeutic drugs employed cause a leukopenia and depress bone marrow. This is particularly true for alkylating agents (e.g., BCNU, Cytoxan, L-phenylalanine mustard, etc.). This effect is similar to that seen occurring in hosts receiving total body irradiation. In both cases, mice receiving non-tolerated doses of alkylating drug (BCNU) or total body irradiation (600-800 r) are spared from death as the result of subsequent levamisole treatment. These observations indicate that the sparing effect is due to stimulation of lymphoproliferation by levamisole.

In homograft tumor and skin test systems, it appears that levamisole exerts an effect on the host that causes earlier and more significant rejection of tumor and skin homografts by stimulating cellular immunity. Data acquired *in vitro* supports the concept that levamisole acts as a mitogen, stimulating more rapid incorporation of labeled thymidine. A significantly higher activity has also been attained by adding levamisole, in nanogram quantities, to an allogeneic mixed lymphocyte culture (MLC).

Three other chemicals demonstrated immunostimulatory activity: polyadenylic, polyuridylic acid (Poly A:U); polyinosinic, polycytidylic acid (Poly I:C); and pyran copolymer. Any of these agents, when used in concert with chemotherapy, demonstrated an additive protective effect apparently due to immunostimulatory activity. These agents, when used alone, do not possess any antitumor activity.

Extracts prepared from a strain of *Staphylococcus aureus* (coagulase negative, penicillin sensitive) have been shown to act as effective adjuvants in leukemic animals placed in remission with chemotherapy. Of four extracts tested, two were found to possess adjuvant activity. Experiments are in progress to compare the activity of intact live and killed *Staphylococcus aureus* with the two active extracts for the purpose of establishing the most effective

biological material. Studies will be conducted with the most active fractions to determine whether their protein or fatty acid components are the responsible moieties for the protective effect attained.

Studies are continuing to determine whether the adjuvant activity demonstrated by interferon when administered to mice during chemotherapeutically-induced remission is mediated through an immunological enhancement or antitumor mechanism.

2. Polymerase inhibitors: Tilorone and several of its analogs have been shown to inhibit the RNA dependent DNA polymerase (RDDP) from the Rauscher leukemia virus (RLV) and avian myeloblastosis virus (AMV). These compounds appear to exert their inhibitory activity by binding with the A-T nucleotide sequences of the various templates used in the enzymatic reaction. In contrast, anthracycline compounds (adriamycin, daunorubicin, and analogs) inhibit the RDDP enzymes by their relative specificity for the viral polymerases. Both classes of compounds, tilorones and anthracyclines, possess *in vitro* and *in vivo* antiviral activity. Our current interpretation of the *in vitro* and *in vivo* antiviral activity of these compounds is that the tilorone compounds exert a 2-fold effect: a diminution of cells replicating virus through non-specific cytoreductive effect; and, a protective effect exerted by endogenous interferon stimulated by tilorones. The anthracyclines exert their effects by inhibiting, non-specifically, cellular DNA synthesis and possibly by binding to RDDP enzyme making it unaccessible for further viral synthesis.

Significance to Biomedical Research and the Program of the Institute:

The multidisciplinary approach represented by this project is almost entirely directed towards the prevention, treatment, and control of cancer. Studies with combined immunotherapeutic modalities have led to a more effective control of neoplasias without an additional burden to the host beyond that imposed by chemotherapy. The advantages of such combined modes of treatment are: (1) no additional cytoreductive chemotherapy is needed to maintain remission; (2) the immune competence of the host is restored allowing host immune mechanisms to function more effectively; (3) maintenance of remission may be retained by intermittent treatment with immunoadjuvants which lacks the limitations inherent with continuous cytoreductive chemotherapy; (4) refractoriness to immunoadjuvants, if it does develop, can be supplemented by additional chemotherapy, with the advantage that the host is in a better physical condition to receive subsequent chemotherapy.

Experimental results conducted with levamisole in this laboratory has led to the inclusion of this agent in chemotherapeutic protocols developed for the treatment of two human tumor types: (1) Dukes' B and C colon and rectal carcinoma which is being presently managed by Surgery and 5-fluorouracil chemotherapy at University Hospitals, Madison, Wisconsin; and (2) non-Hodgkin's lymphoma managed by combined chemotherapy by the Medicine Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland. This Section will be collaborating with the University Hospitals and the NCI Medicine Branch in monitoring the efficacy of levamisole immunostimulation in cancer patients.

Proposed Course:

Each of the areas described under objectives will be pursued. Emphasis will be placed on quantitative and qualitative studies in three specific areas, i.e., immunostimulators, combined treatment modalities, and oncornavirus inhibitors. Biochemical parameters will be investigated with the active inhibitors to assess mechanism(s) of action. Immunostimulation studies with chemical and biological meterials will be extended to carcinogen-induced and spontaneously-occurring tumors and to other tumors already described to determine effectiveness. The role of interferon in protracting the remission period induced in leukemic mice by initial drug therapy will be investigated in more detail. Additional *in vitro* and *in vivo* methods will be implemented to further define the mechanism of action of the active chemical and biological immunostimulators in terms of T-cell, B-cell, or macrophage function.

Honors and Awards:

None

Publications:

Chirigos, M. A. and Papas, T. S.: Immunological and chemotherapeutic prevention and control of oncogenic viruses. *Advances in Pharmacology and Chemotherapy* 12: 1-35, 1975.

Chirigos, M. A., Fuhrman, F., and Pryor, J.: Prolongation of chemotherapeutically induced remission of a syngeneic murine leukemia by levamisole. *Cancer Res.* (In Press, April 1975).

Chirigos, M. A., Mohr, S. J., and Chaparas, S. D.: Combined modality treatment with levamisole against neoplasias and protective effects. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Papas, T. S., Pry, T. S., and Chirigos, M. A.: Inhibition of RNA-dependent DNA polymerase of avian myeloblastosis virus by pyran copolymer. *Proc. Natl. Acad. Sci.* USA, 71: 367-370, 1974.

Papas, T. S., Chirigos, M. A., and Chirikjian, J. G.: Protection of avian myeloblastosis virus (AMV) DNA polymerase by substrates against heat inactivation. *Nucleic Acids Research* 1: 1399-1410, 1974.

Papas, T. S., Chirikjian, J. G., Pry, T. W., Massicot, J., Irwin, R. D., and Chirigos, M. A.: Effect of chemically modified 70S RNA from avian myeloblastosis virus (AMV) upon the activity of AMV DNA polymerase. *J. Virol.* 14: 1108-1114, 1974.

Schafer, M. P., Chirigos, M. A., and Papas, T. S.: Inhibition of Rauscher leukemia virus and avian myeloblastosis virus DNA polymerase by tilorone and its analogs. *Cancer Chem. Repts.* 58: 821-827, 1974.

Pearson, J. W., Chaparas, S. D., Sher, N. A., and Chirigos, M. A.: Combined drug and immunostimulation therapy against a syngeneic murine leukemia. *J. Nat. Cancer Institute* 51: 463-468, 1974.

Perk, K., Pearson, J. W., Torgersen, J. A., and Chirigos, M. A.: An animal model for meningeal leukemia. *Int. J. Cancer* 13: 863-866, 1974.

Chaparas, S. D., Chirigos, M. A., Pearson, J. W., and Sher, N. A.: BCG immunization for the lymphoid leukemia lines (LSTRA) in CDF1 mice. *National Cancer Institute Monograph* 39: 87-88, 1974.

Woods, W. A., Siegel, M. J., and Chirigos, M. A.: *In vitro* stimulation of spleen cell cultures by poly I:poly C and levamisole. *Cellular Immunology* 14: 327-331, 1974.

Perk, K., Chirigos, M. A., Fuhrman, F., and Pettigrew, H.: Brief communication: Some aspects of host response to levamisole following chemotherapy in a murine leukemia. *J. Natl. Cancer Institute* 54: 253-256, 1975.

Woods, W. A., Fliegelman, M. J., and Chirigos, M. A.: Effect of levamisole on the *in vitro* immune response of spleen lymphocytes. *Proc. Soc. Exper. Biol. and Med.* (In Press, 1975).

Woods, W. A., Papas, T. S., and Chirigos, M. A.: The effect of levamisole on the *In vitro* parameters of the immune response. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Papas, T. S., Chirikjian, J. G., Woods, W. A., and Chirigos, M. A.: Active site probes and the selective inhibition of RNA tumor viral DNA-polymerase. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Pearson, J. W., Perk, K., Chirigos, M. A., and Torgersen, J. A.: Drug therapy against a transplantable guinea pig leukemia. *Cancer Research* (In Press, 1975).

Price, P. J., Suk, W. A., Skeen, P. C., Chirigos, M. A., and Huebner, R. J.: Transforming potential of the anticancer drug andriamycin. *Science* (In Press, 1974).

Mohr, S. J. and Chirigos, M. A.: Tilorone hydrochloride as immunoadjuvant therapy against murine neoplasia. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Serial No. Z01-CP-04876-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Alterations Occurring in Host Tissue as a Result of Oncogenic Virus Infection and Tumor Induction

Previous Serial Number: SAME

Principal Investigator: Dr. Paul S. Ebert

Other Investigators: None

Cooperating Units: Inside NIH
Dr. G. F. Vande Woude, VBB, NCI
Dr. J. Simonds, VBB, NCI
Dr. D. N. Buell, IB, NCI
Dr. D. P. Tschudy, MB, NCI
Dr. J. Minna, BG, NHLI
Ms. B. J. Fowlkes, LP, NCI

Outside NIH:
Dr. J. R. Bloomer, Yale University
New Haven, Connecticut
Dr. H. L. Bonkowsky, V.A. Hospital
White River Junction, Vermont

Man Years:

Total:	2.2
Professional:	1.1
Other:	1.1

Project Description

Objectives:

1. To determine the significance of the heme biosynthetic pathway in RNA tumor virus-producing non-erythroid cell lines.

2. To study the interrelationships between malignancy, virus production and differentiation in Friend virus leukemia cells which can be stimulated to differentiate along the erythroid pathway by exposure to dimethylsulfoxide (DMSO).

3. To delineate biochemical defects in the genetic disease acute intermittent porphyria.

Methods Employed:

Conventional biochemical procedures were utilized to follow alterations occurring in cultured cells. Virus particles and viral RNA were concentrated and purified by centrifugation and standard density gradient techniques. Heme synthesis was monitored by following increases in δ-aminolevulinic acid synthetase (ALAS) activity and hemoglobin production. ALAS activity was measured by a radiochemical chromatographic procedure. Hemoglobin was measured using a sensitive colorimetric method involving the benzidine reaction. Reverse transcriptase was measured by disrupting concentrated virus particles with detergent, incubating the lysates with ^{3}H-TTP and ^{3}H-GTP and rA·dT or rC·dG templates respectively, and collecting the acid-precipitable products of the reaction on Millipore filters. Thymidine kinase and DNA-dependent DNA polymerase were measured by radiochemical techniques.

Major Findings:

1. Heme biosynthesis and reverse transcriptase activity in Friend leukemia cell lines: A proerythroblastoid mouse cell line, T3C12, which chronically releases Friend virus (FV) can be induced to synthesize hemoglobin upon treatment with such agents as dimethylsulfoxide (DMSO), dimethylformamide, mitomycin C, and adriamycin. A reduction in reverse transcriptase (RT) activity of released FV preceded an increase in the activity of the control enzyme of the heme pathway, ALAS, and hemoglobin production in DMSO-treated cells. The decreased RT activity is accompanied by an increase in the amount of 60-70S RNA synthesized, suggesting that DMSO stimulates the production of FV deficient in RT.

Friend had observed inhibition of DNA, RNA, and protein synthesis within 24 hours after administration of DMSO to cells. Compounds such as cytosine arabinoside, methylurea, mithramycin, and cycloheximide are known to selectively inhibit a single synthetic pathway and individually they show minimal stimulation of hemoglobin synthesis in T3C12 cells. However, combinations of methylurea:mithramycin, or methylurea:cycloheximide, or methylurea:mithramycin are as effective as DMSO in stimulating erythroid differentiation. Modest inhibition of DNA synthesis and, in addition, partial inhibition of RNA and/or protein synthesis appear to be required to trigger hemoglobin synthesis. Partial inhibition of RNA and protein synthesis in the absence of DNA synthesis did not result in any substantial hemoglobin synthesis.

A recent report stated that 2 cycles of cell division in a Friend clone were necessary before erythroid differentiation could proceed. T3C12 cells in stationary phase were subjected to DMSO or methylurea:cycloheximide treatment at daily intervals after splitting cell cultures. Appreciable hemoglobin production was attained in methylurea:cycloheximide-treated cells which did not enter the log phase of cell division. Erythroid differentiation was only

initiated in cells which were treated with drugs before or at the point when the cells entered the log phase of growth. Drugs administered during the log phase were ineffective in stimulating differentiation.

Friend's clone 745 was compared to the T3C12 clone. RT activity/cell was 15-25 times higher in clone 745 and the rate of cell replication was higher in comparison to T3C12 cells. Both the basal activity and the DMSO-induced level of ALAS activity in hemoglobin production was also less in the latter.

2. Enzyme changes in cultured fibroblasts from patients with erythropoietic protoporphyria: In collaboration with H. L. Bonkowsky we have investigated biochemical abnormalities in cultured skin fibroblasts from 8 patients with erythropoietic protoporphyria (PP) and from 4 normal subjects. Activity of the first limiting enzyme in the heme biosynthetic pathway, ALAS, was nearly the same in the 2 types of cells, whereas ferrochelatase (FC) activity was much lower in the cells from patients with PP than in those of controls. The decrease in FC is considered important in the pathogenesis of PP since it would lead to protoporphyrin accumulation by cells whose heme requirement exceeded the FC activity, if the FC activity is the limiting step in the porphyrin synthetic pathway.

Significance to Biomedical Research and the Program of the Institute:

Investigations of early metabolic changes during the differentiation of FV-transformed cells to red cells can lead to a better understanding of relationships between malignancy and differentiation. The relative ability of different classes of drugs to mistake erythroid differentiation in this model system can provide an improved undertanding of drug action and also help elucidate the control mechanisms for erythroid differentiation. This model system might also be developed into a drug screening method for drugs which can modify certain types of malignancy and thus promote cellular differentiation.

Studies on the heme biosynthetic activity of virus-producing, nonerythroid cell cultures can determine the significance of the increased demand for heme-containing compounds in these cells.

Proposed Course:

Clones of Friend leukemia cells will be further studied to define the factors which modify malignancy and control red cell differentiation. Relationships between phases of the cell cycle and the initial requirement for inhibition of DNA-RNA-protein synthetic pathways of these cells to trigger hemoglobin synthesis need to be established.

Attempts will be made to determine the relationship between tumor virus production and heme synthesis in non-erythroid cells and which heme-containing compounds, such as enzymes and cytochromes, are required by the virus-producing cell.

Honors and Awards:

None

Publications:

Ebert, P.S. and Ikawa, Y. Induction of δ-aminolevulinic acid synthetase during erythroid differentiation of cultured leukemia cells. *Proc. Soc. Biol. and Med.* 146: 601-604, 1974.

Bonkowsky, H.L., Tschudy, D.P., Weinbach, E.C., Ebert, P.S. and Doherty, J.M. Porphyrin synthesis and mitochrondrial respiration in acute intermittent porphyria: Studies using cultured human fibroblasts. *J. Lab. & Clin. Med.* 85: 93-102, 1975.

Serial No. ZO1-CP-04877-06-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Virus and Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modification of the Normal and Neoplastic Cell by Oncogenic and Non-oncogenic Viruses

Previous Serial Number: SAME

Principal Investigator: Dr. Wilna A. Woods

Other Investigators: Dr. Michael A. Chirigos
Dr. John W. Pearson
Dr. Takis S. Papas
Dr. Martin J. Fliegelman
Dr. Stephen J. Mohr

Cooperating Units: Inside NIH:
Dr. S. Chaparas, DBP, BB

Outside NIH:
Dr. A. Garretson, Frederick Cancer Research Center, Frederick, Maryland
Dr. R. Sonstegard, University of Guelph, Guelph, Ontario, Canada
Dr. E. Borden, University Hospitals, University of Wisconsin, Madison, Wisconsin

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To investigate intracellular interactions between oncogenic and non-oncogenic viruses *in vitro*.

2. To investigate alteration of antigenic composition of tumor cell membranes by infection with non-oncogenic viruses.

3. To investigate use of tumor cells with altered membrane antigenic profiles for use in immunotherapy and prophylactic vaccination.

4. To investigate the effect of chemotherapeutic agents on RNA tumor virus replication *in vitro*.

5. To determine by *in vitro* techniques the mechanism of action and optimal treatment schedules of various immune enhancer drugs for application to the treatment of neoplasms.

Methods Employed:

A. *In vitro* systems:

1. Cell lines of murine and human origin have been established and characterized. These lines can now be modified experimentally for basic immunologic and virologic studies.

2. ^{3}H-uridine incorporation into particles banding at 1.16 g/ml in a sucrose density gradient centrifugation is being used to identify presence of RNA in viral particles and demonstrate the presence of viral particles in cells.

3. Radioisotope procedures for detecting and characterizing RNA, DNA, and protein synthesis are now established on a routine basis.

4. Routine procedures include virus titrations using focus formation by the Moloney virus pseudotype of murine sarcoma virus (MSV-M), X-C plaque and lytic plaque formation, and fluorescent focus assay. Antigenic determinations are made by methods including fluorescent antibody staining, cytotoxicity, virus neutralization, and hemagglutination inhibition.

5. Tests for monitoring cellular immune responses include mixed lymphocyte reactions, PHA stimulation, lymphocyte-mediated cytotoxicity, and serum blocking factors.

B. *In vivo* systems:

1. BALB/c mice are used in MSV-M studies.

2. L1210 bearing DBA, C57Bl, and BALB/c mice are being used to determine immune responses to a non-virus induced tumor.

3. MBL (MSV-M induced C57Bl tumor) in C57Bl and BALB/c mice is being used to determine immune reactions to a virus-induced tumor.

Major Findings:

The mechanism of action of the immunostimulatory chemical levamisole (LMS) was determined to be a non-specific mitogenic activity on resting lymphoid cells. No mitogenic activity on fibroblasts, macrophages, or tumor cells was seen. The stimulation was apparently mediated by the activity of the drug on the cGMP-cAMP "second messenger" complex via interaction with phosphodiesterase.

Stimulation of the immune system by chemical agents such as poly I:poly C, polyanions, tilorone, pyran copolymer, and levamisole have been demonstrated by *in vivo* methods. The chemicals poly I:poly C and levamisole are active as *in vitro* stimulators of spleen cell cultures, and levamisole has been shown to be a stimulator of peripheral blood lymphocytes. We have used the *in vitro* mixed lymphocyte reaction to demonstrate a direct stimulatory action of levamisole on cells involved in allogeneic recognition reactions and report here that low concentrations of levamisole induce blastogenic stimulation of spleen cell cultures as well as enhance allogeneic stimulation in mixed lymphocyte cultures.

The effect of *in vivo* treatment of C57Bl mice with BCNU [1,3-bis(2-chloroethyl) 1-nitrosourea] and or LMS on the *in vitro* DNA synthetic capacity of their spleen cells was studied as a measure of cell-mediated immune function. BCNU treatment was suppressive to spleen cell DNA synthesis; conversely, treatment with LMS was stimulatory. LMS treatment 5-8 days after BCNU treatment resulted in significant recovery of DNA synthetic capacity. Multiple doses of LMS were not more effective than single doses. Allogeneic stimulation of BCNU-suppressed lymphocytes was not consistently increased by LMS treatment.

Sera from 8 of 15 patients with colonic carcinoma exhibited demonstrable cytotoxicity against an established homogenous cell strain derived from adenocarcinoma of the ileocecum, HCT-8. Sera from 12 of 16 patients with rectal carcinoma were cytotoxic for an established homogenous cell strain derived from an adenocarcinoma of the rectum, HRT-18. Patients with colonic carcinoma exhibited serum cytotoxicity against only the colonic target cells, whereas patients with rectal carcinoma gave significant cytotoxicity against both cell strains. This cytotoxicity was shown to be complement-dependent and appeared to be specific for colonic and/or rectal carcinoma cells. Although the cells produced carcinoembryonic antigen, CEA, *in vitro*, the cytotoxic antibody response in these patients did not appear to be directed against CEA. Serum cytotoxicity was not demonstrated against two other cell strains, HCT-48 and HT-29, derived from adenocarcinomas of the human colon, except for a reaction against a blood-group-related antigen. These cell strains had comparable levels of cell-associated CEA. Our results suggest the presence of an additional common antigen in adenocarcinomas of the rectum. The routine titration of cytotoxic antibody against these established cell cultures may provide meaningful information on the host's immune response to colorectal neoplasms.

Peripheral blood lymphocytes from colorectal carcinoma patients and from patients with other entodermally-derived malignancies inhibited colony formation by established cell strains of adenocarcinoma of the ileocecum, HCT-8; adenocarcinoma of the rectum, HRT-18; and human embryonic intestine, HEI. Abrogating factors of this cell-mediated immunity could be demonstrated in serum from patients wtih advanced carcinomas of the colon, esophagus, liver, rectum, and stomach but not from patients with nonentodermal cancers. These enhancing factors were also utilized to distinguish between cell-mediated immune reactions directed against transplantation and tumor-specific antigens on these cultured allogeneic cells. Specificity was demonstrated by the fact that a pool of sera which antagonized the colony inhibition by lymphocytes from patients with entodermally-derived neoplasms did not abrogate the reactivity of lymphocytes from two control donors. The tumor-associated immunity is probably directed against a re-emergent embryonic or fetal antigen distinct from carcinoembryonic antigen, CEA, because CEA could not be detected in the HEI cultures and because the relative amounts of CEA synthesis by the target cells did not correlate with the degree of colony inhibition by sensitized lymphocytes. Furthermore, the addition of purified CEA did not exert any demonstrable effect on lymphocyte reactivity. These established cell cultures offer a standardized *in vitro* system in which the various parameters of the host's immune reactions can be monitored and correlated with the clinical events occurring for an objective determination of prognosis in patients undergoing therapy.

Inhibition of Rauscher leukemia virus and avian myeloblastosis virus DNA polymerases by tumor cell extracts of human colorectal carcinoma: Virus-like particles have been previously observed by electron microscopy in early passages of HCT-8 cells. An attempt was made to detect possible oncornavirus DNA polymerase activity in tumor cell extracts from cultured colorectal cells. In the course of these studies, a potent inhibitor of RNA-dependent DNA polymerase was observed and partially characterized. This extracted material did not inhibit bacterial (*M. luteus*) or cellular (JLSV-9) DNA polymerases. It could be recovered in the supernatant after centrifugation at 20,000 RPM for 60 minutes. It was not dialyzable; it was neither a ribonucleic acid nor ribonuclease. It was partially inactivated when held for 30 minutes at 60C and completely inactive after 30 minutes at 80C. This inhibitor was not found in extracts of Hela, A1-2, or human skin fibroblasts.

MBL-2 Homograft Response: CDF1 mice lose their ability to reject a homograft of MBL-2 cells following treatment with pyran. A cell line, established from the ascites transplant line of MBL-2, grows in suspension culture. It is in its 40th sub-culture. These cells are tumorigenic in C57Bl with a $TD_{50}<100$ cells. Using the cultured cells as targets, a study is being undertaken to determine pyran's effects on the immune system. A ^{51}Cr microtechnique has been developed to quantitate lymphocyte mediated cytotoxicity, humoral blocking factors, and complement-dependent cytotoxic antibody. Although preliminary, our results suggest that pyran may increase serum blocking factors and/or decrease lymphocyte-mediated cytotoxicity. Sequential studies are underway to determine the importance of these changes to tumor control by the host.

Two lines of mouse leukemia, L1210 and MBL, were shown to inhibit DNA synthesis of normal mouse spleen lymphocytes in mixed culture. Target leukemia cells inactivated by two methods were used, mitomycin C and ultraviolet irradiation. The inhibitory effect was present with both methods, indicating that free mitomycin C carried into the mixed culture by the target cells was not responsible for the inhibition. Addition of inactivated MBL cells, but not of inactivated syngeneic or allogeneic normal lymphocytes, inhibited DNA synthesis in mixed lymphocyte culture. An extract of the tumor cells, but not of normal lymphocytes, prepared by freezing and thawing, also inhibited the MLR. The activity was present in both membrane and "soluble" fractions of the extract. Further identification studies are underway.

Various fish tumor cells were provided by Dr. R. Sonstegard and examined for viral reverse transcriptase activity. An extract of pike lymphoma, brown bull head plasma cytomay, muskelunge lymphoma were found to contain viral-like RNA directed DNA polymerase activity which had an optimum temperature of 20C and was inactive at 37C. The activity could not be transmitted to tissue cultures of bluegill-free, trout gonod.

Significance to Biomedical Research and the Program of the Institute:

The study of the interaction between oncogenic virus and non-oncogenic virus may be of importance in determining the "trigger" mechanism for tumor induction in animals (including man) carrying an oncogenic virus genome from conception.

The study of the mechanisms of action of potentially therapeutic drugs on both cell and virus replication has value for screening such agents *in vitro* before application *in vivo*.

The study of ^{3}H-uridine incorporation into higher molecular weight RNA of malignant cells is of importance for the detection of cells bearing defective viral genomes.

The *in vitro* studies of cellular and humoral immune responses are important for identifying potential immunostimulatory agents and measuring their mechanism(s) of action. *In vitro* assays are also capable of efficiently determining the effect of immunostimulatory drugs on the immune suppression of host defenses imposed by tumor and chemotherapy. The relationship of treatment schedules of combined chemotherapeutic-immunotherapeutic regimens to restoration of immune responses can also be determined.

The colon carcinoma cell lines can be used (1) to monitor patients' immune responses to tumor antigens during the course of their disease and therapy, enabling the clinican to objectively determine the efficacy of therapy and also allowing correlation of the various immune parameters with prognosis and/or diagnosis, (2) to determine the nature of the various tumor or organ specific antigens and the relation of the response to them to prognosis, (3) to allow purification of those antigens apparently correlated with the host immune regression of the tumor and use of such purified antigens for

prophylaxis, and (4) to determine the nature of the serum factor which seems to abrogate cell-mediated cytolysis.

Proposed Course:

1. The effect of LMS and other immune stimulators on the specific host-tumor response will be monitored by sequential specimens from patients undergoing chemoimmunotherapy, as well as in animal model systems.

2. The interaction of LMS and other potential stimulators with the cAMP-cGMP regulatory enzymes will be pursued.

3. The immune cell inhibitory factors in the T cell leukemias of mice will be biochemically identified.

4. The inhibitory factor for reverse transcriptase in colon ca cells will be further characterized.

5. Antigens of the colorectal ca cell lines will be identified with specific immune parameters.

6. The sequential immune responses to colorectal tumors will be determined in both animal models and patients.

7. Cell lines will be developed to allow immunological studies of other tumor types.

Honors and Awards:

None

Publications:

Woods, W. A., Siegel, M. J., and Chirigos, M. A.: *In vitro* stimulation of spleen cell cultures by poly I:poly C and levamisole. *Cellular Immunology* 14: 327-331, 1974.

Woods, W. A., Fliegelman, M. J., and Chirigos, M. A.: Effect of levamisole on the *in vitro* immune response of spleen lymphocytes. *Proc. Soc. Exper. Biol. and Med.* (In Press, April 1975).

Woods, W. A., Papas, T. S., and Chirigos, M. A.: The effect of levamisole on *in vitro* parameters of the immune response. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Papas, T. S., Chirikjian, J. G., Woods, W. A., and Chirigos, M. A.: Active site probes and the selective inhibition of RNA tumor viral DNA polymerase. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Serial No. Z01-CP-04878-06-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Virus and Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical, and Physical Methods

Previous Serial Number: SAME

Principal Investigator: Dr. John W. Pearson

Other Investigators: Dr. Michael A. Chirigos
Dr. Wilna A. Woods
Dr. Takis S. Papas
Dr. Stephen J. Mohr

Cooperating Units: Inside NIH:
Dr. Gary Pearson, VBB, NCI
Dr. John Dahlberg, ET, NCI
Dr. Robert Oldham, LCBGY, NCI
Dr. Sotoros Chaparas, DBP, BB

Outside NIH:
Dr. Joseph Coggin, AEC, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Dr. Kalman Perk, The Hebrew University of Jerusalem, Rehovot, Israel

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

The overall goal of this project is the achievement of effective therapeutic control of spontaneous and transplantable leukemias and lymphomas. The therapeutic measures encompass individual or combination drug therapy, surgery, immunotherapeutic methods, vaccines, and the application of interferon. Any therapy leading to effective control will be submitted for preclinical testing, evaluation, and eventual clinical trial.

Methods Employed:

Mouse Virus and Tumor Systems:

1. The Friend, Rauscher, Moloney leukemogeneic viruses, the Moloney sarcoma virus, and the plasma passaged virus varient (MSV-PV) are used for *in vivo* studies.

2. Transplantable tumors used are: Moloney lymphoid leukemia ascites cell lines (LSTRA and MCAS-10), a transplantable tumor cell line induced by Graffi leukemia virus, a Gross virus-induced transplantable tumor cell line, a reticulum cell sarcoma ascites line, a transplantable Lewis lung carcinoma cell line and a transplantable mammary carcinoma cell line.

3. AKR and C3H/HEN mice which have a high incidence of spontaneous neoplasia are used for *in vivo* tests.

4. Rat transplantable tumors are presently under investigation *in vivo*. These include: (a) a Moloney lymphoid leukemia ascites line; (b) a transplantable ascites Gross leukemia line and (c) a Bogden mammary carcinoma transplantable tumor line.

5. A transplantable virus-associated Gross leukemia in strain 2 guinea pigs is currently being employed for *in vivo* studies.

6. Surgical techniques are now being applied to remove the primary tumor at the initial site of inoculation.

7. Two mitogens, PHA and Con A, are presently being utilized *in vitro* to attempt to monitor for drug suppression following intensive drug therapy against diseased animals.

8. Soluble tumor-specific antigens (TSA) are being prepared by (3M) KCl extraction procedures to monitor delayed-type cutaneous hypersensitivity responses in rat and guinea pig tumor systems.

Major Findings:

Chemoimmunostimulation Therapy: A Graffi murine leukemia was utilized as a model system to investigate the effect of chemoimmunostimulation therapy in mice. The disease is characterized as a rapidly growing tumor at the site of inoculation with subsequent development of splenomegaly and lymphoadenopathy. All animals succumb to the leukemia within 24 to 30 days. Treatment of diseased animals with 2 courses of cytoxan over a 2-week period resulted in a remission period of approximately 16 to 18 days before relapse and eventual death of approximately 70% of the drug-treated animals. A significant number of long-term survivors (50 to 80%) were obtained in groups of animals that received combined drug plus BCG or *C. parvum* therapy. In contrast, the administration of MER (methanol extracted residue of tubercule bacilli of the

Phipps strain of BCG) to animals in a drug-induced remission period was not more effective than drug alone. The protective effect afforded by BCG and *C. parvum* was dependent on the time interval between drug therapy and the administration of the immunostimulators. The protective effect of BCG appeared to be independent of route of administration. No leukemic cells were observed in any of the histologically examined tissues taken from long term survivors.

Drug studies against a transplantable mammary carcinoma in C3H/HEN mice have been in progress. Such clinically active drugs as cytoxan, methyl CCNU, adriamycin, 5 FU, MTX, and vincristine used alone or in combination have failed to control the established tumor. Studies are currently underway to apply surgery for the removal of the primary tumor at a time when metastasis has occurred to either the liver or lungs. The efficacy of various forms of immunotherapy will then be applied to control the metastastic lesions.

Histological and drug therapy studies are continuing in collaboration with Dr. Perk, The Hebrew University, against a transplantable virus-associated Gross leukemia in strain 2 guinea pigs. Treatment of leukemic animals with cytoxan weekly for two weeks resulted in prolongation of survival time associated with disappearance of the tumor mass at the site of inoculation, a decrease in the degree of organ involvement and a normal blood picture. On "relapse," characterized by an increase in WBC counts and reappearance of blast cells in the peripheral blood, most animals showed clinical central nervous system (CNS) signs, i.e. paresis, paralysis, and convulsions which led to prostration and eventual death. Histological examination of brain sections obtained from cytoxan-treated guinea pigs at different stages during the course of the disease revealed that the CNS leukemia was present in the superficial and deep arachnoid tissue of the brain. A two-drug combination of cytoxan and methyl CCNU was found not only to prevent meningeal leukemia development, but to "cure" all animals from their leukemia. This observation was based on a complete clinical, hematological, and histopathological "remission" period up to 176 days. The administration of methyl CCNU alone was observed not only to control systemic leukemia but to prevent CNS involvement. Results indicate the methyl CCNU should be considered for incorporation in the treatment modality currently used against acute lymphocytic leukemia in man. Various forms of immunotherapy are currently being applied to animals in "remission" following intensive cytoxan therapy in hopes of preventing meningeal leukemia. In addition, soluble antigen preparations obtained from tumor and leukemic blast cells are being administered *in vivo* to monitor the delayed-type cutaneous hypersensitivity response following immunostimulation therapy.

In collaboration with Dr. John Dahlberg, studies have shown the presence of the RNA-dependent DNA polymerase (reverse transcriptase) in plasma obtained from leukemic guinea pigs. Studies are currently underway to monitor for the presence of this enzyme in animals following cytoxan or methyl CCNU therapy. It is conceivable that the absence of this enzyme during a drug-induced "remission" as well as its subsequent recurrence at "relapse" may serve as an additional parameter to monitor the course of this leukemia.

The Bogden mammary carcinoma transplantable tumor line in Fischer rats has been under study this year. Of all the clinical drugs screened against this tumor, cytoxan has shown to be the most effective in inducing a remission period (6 to 8 weeks) before eventual relapse and death of 90% of the animals. Surgical removal of the primary tumor (20 to 25 mm) resulted in subsequent metastases to the axillary nodes and lungs with death occurring approximately 3 weeks after surgery. Various forms of immunotherapy are now being applied following cytoxan therapy or surgery to control the metastastic lesions. *In vivo* (soluble antigens) and *in vitro* (Chromium 51) tests are being used to monitor the animals' immune response following immunostimulation therapy. Similar studies are in progress involving a transplantable virus-associated Gross leukemia in WF/u rats.

Significance to Biomedical Research and the Program of the Institute:

These studies are directed toward the prevention, treatment, and control of virus-induced or spontaneous leukemias, lymphomas, and sarcomas in a variety of animal systems. Comparisons made by the use of drugs, alone and in combination with immunostimulators, immunotherapy, and vaccines may lead to more effective therapeutic measures.

Proposed Course:

Research in each of the areas described under objectives will continue. More emphasis will be placed in maintaining prolonged periods of remission following chemotherapy or surgery. The use of immunostimulators alone and in combination with syngeneic and allogeneic vaccines as well as interferon will be investigated in mouse, rat, and guinea pig leukemia and tumor systems. Similar approaches will be attempted against the spontaneous AKR and C3H/HEN neoplasms. Immunization procedures will be initiated in order to determine the antigenicity of various tumor lines. The mechanism of action of immune stimulators are now being used and/or new ones that evolve will be studied by *in vitro* and *in vivo* techniques. It is hoped that information gained from these investigations may be applied toward the control of human cancer.

Honors and Awards:

None

Publications:

Pearson, J. W., Chaparas, S. D., Sher, N. A., and Chirigos, M. A.: Combined drug and immunostimulation therapy against a syngeneic murine leukemia. *J. Nat. Cancer Institute* 51: 463-468, 1974.

Perk, K., Pearson, J. W., Torgersen, J. A., and Chirigos, M. A.: An animal model for meningeal leukemia. *Int. J. Cancer* 31: 863-866, 1974.

Chaparas, S. D., Chirigos, M. A., Pearson, J. W., and Sher, N. A.: BCG immunization for the lymphoid leukemia lines (LSTRA) in CDF1 mice. *National Cancer Institute Monograph* 39: 87-88, 1974.

Pearson, J. W., Perk, K., Chirigos, M. A., and Torgersen, J. A.: Drug therapy against a transplantable guinea pig leukemia. *Cancer Research* (In Press, 1975).

Serial No. Z01-CP-04879-02-VB

1. Viral Biology Branch
OADVO, Division of Cancer
Cause and Prevention
2. Virus and Disease Modification
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1974 through June 30, 1975

Project Title: *In Vitro* Assessment of Immunomodulation

Previous Serial Number: SAME

Principal Investigator: Dr. Martin J. Fliegelman

Other Investigators: Dr. Wilna A. Woods
Dr. Michael A. Chirigos

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To develop an *in vitro* assay of lymphocyte stimulation and inhibition by immune stimulators and repressors.

2. To determine the mechanism of action of the immune stimulators and repressors.

3. To test new drugs for *in vitro* immune stimulatory and repressive activity.

4. To apply the knowledge obtained about the mechanism of action to develop new drugs that would be more active in the treatment of cancer.

5. To isolate and purify a factor from tumor cells that inhibits the multiplication of normal lymphocytes.

6. To determine if immune stimulators can hasten the return of immune competence.

7. To determine if levamisole works through a β adrenergic receptor.

Methods Employed:

A. *In vivo* systems:

1. Splenic lymphocytes from mice are put into short-term cell culture by different methods. The incorporation of ^{3}H-TdR by these cells is measured after stimulation by phytohemagglutinin, pokeweed mitogen, immune stimulator drugs and combination of the three.

2. Murine splenic lymphocytes are put into short-term culture. The target cells are incubated with mitomycin C to block DNA synthesis. The effector and target cells are mixed at a 1:2 ratio and cultured together for 96 hours. The incorporation of ^{3}H-TdR by the effector cells is measured in response to various target cells, and the effect of several drugs and extracts on this reaction is observed.

3. Murine splenic lymphocytes are put into short-term culture. The target cells are labeled with ^{51}Cr. The effector and target cells are mixed at a 10:1 ratio and incubated for 4 hours. The release of ^{51}Cr into the medium is measured as an estimation of target cell damage.

4. H-isoproterenol is bound to murine erythrocyte ghosts; then unlabeled levamisole is added to competitively remove the isoproterenol. The cell membranes are trapped on a Millipore filter, and the radioactivity determined.

B. *In vivo* then *in vitro* systems:

1. Mice are injected i.p. with immune stimulator drugs. The mice serve as donors for lymphocytes in the *in vitro* system described.

Major Findings:

The antihelminthic drug, L-2,3,5,5-tetrahydro-6-phenyl-imidazo [2,1-b] thiazole hydrochloride [Levamisole (LMS)] possesses immunostimulatory activity in several bacterial and tumor systems. We have shown that DNA synthesis in normal murine lymphocytes alone, or in mixed lymphocyte culture (MLC), is stimulated by LMS. We report here that chemotherapeutically-induced immunosuppression can be overcome by treatment with LMS. C57Bl/6 and DBA/2 mice were immunosuppressed with BCNU [1,3-bis (2-chloroethyl)-1-nitrosourea], then treated with LMS and at various times after LMS treatment spleens were removed for MLC. LMS treatment alone resulted in enhanced DNA synthesis by unstimulated lymphocytes of both strains. Cells from BCNU-suppressed C57Bl/6 mice treated with LMS on day 1 or later had returned to normal DNA synthetic levels within 7 days of LMS treatment. Conversely, lymphocytes from immunosuppressed DBA mice were not stimulated. Allogeneic stimulation of cells derived from animals treated with LMS alone had higher overall thymidine incorporation than cells from controls, reflecting stimulation of lymphocytes alone. BCNU-treated C57Bl/6 animals were capable of responding to allogeneic stimulation 5-8 days after treatment with LMS, in contrast to the immuno-

suppression observed through day 21 due to BCNU treatment alone. DBA mice were capable of significant response to allogeneic stimulation 8 days after BCNU treatment; LMS treatment overcame the suppression before day 8 and enhanced the response thereafter.

Stimulation of the immune system by chemical agents such as poly I:poly C, polyanions, tilorone, pyran copolymer, and levamisole has been demonstrated by *in vivo* methods. The chemicals poly I:poly C and levamisole are active stimulators of cultured spleen cells, and levamisole has been shown to stimulate peripheral blood lymphocytes. We have used the mixed lymphocyte reaction to demonstrate a direct stimulatory action of levamisole on cells involved in allogeneic recognition reactions and report here that low concentrations of levamisole induce blastogenesis in cultured spleen cells as well as enhance allogeneic stimulation of cells in mixed lymphocyte cultures.

The effect of the treatment of C57Bl mice with BCNU and/or LMS on the DNA synthetic capacity of their spleen cells *in vitro* was studied as a measure of cell-mediated immune function. BCNU treatment suppressed spleen cell DNA synthesis; conversely, treatment with LMS was stimulatory. LMS treatment 5-8 days after BCNU treatment resulted in significant recovery of the capability the cells to synthesize DNA. Multiple doses of LMS were not more effective than single doses. Response to allogeneic stimulation by BCNU suppressed lymphocytes was not consistently increased by LMS treatment.

L1210, a spontaneous DBA T-cell tumor, and MBL-2, a Moloney virus-induced C57Bl/6 T-cell tumor, have been shown to be unreactive as target cells in the mixed lymphocyte culture (MLC) assay. In fact, they have been shown to inhibit a normal proliferative response by other cells in a 3-day MLC test. However, they do not cause ^{51}Cr release above background in the chromium release assay, so the effect is not due to cell killing. Further, a crude extract of MBL-2 cells has been shown to be equally inhibitory, while an extract from normal C57Bl/6 lymphocytes had no effect.

As part of this laboratory's investigations with levamisole (LMS), studies were undertaken to determine if LMS, a suspected activator of cyclic AMP, binds to the membrane at a β-adrenergic receptor. The methods used were an adaptation of Aurback's competitive binding to erythrocyte ghosts. The experiments did not indicate that LMS binds to β-receptors. However, it has since been shown that the method is faulty. The isoproterenol was binding to the membrane-bound, degredative enzyme, catechol-O-methyl-transferase, not to β receptor. To detect the true β receptor will require isoproterenol of much higher specific activity, so the project was abandoned until a later date when the methodology will be further refined.

Significance to Biomedical Research and the Program of the Institute:

There is presently much interest in the field of tumor immunology in defining the relationship of cell-mediated and humoral immunity to the establishment and repression of neoplasms. A variety of immune stimulators have been shown to prolong drug-induced remission in mouse tumor systems, resulting in

increased survival of the animals. An *in vitro* screening assay of drugs for immune stimulator activity would speed up the search for such drugs and help elucidate the mechanism of their action.

Proposed Course:

1. To define the nature of the MBL-2 tumor extract and purify it. It is felt that this extract might be related to tumor specific transplantation antigen (TSTA) or another tumor-related cell surface antigen. Hence, an antibody to this extract might reverse this "immunosuppression" and enable normal cell-mediated immunity to destroy the tumor.

2. To determine if B-cell tumors respond differently in MLC than the T-cell tumors already studied.

3. To determine the contribution of B- and T-lymphocytes in both the tumor extract and MLC system.

Honors and Awards:

None

Publications:

Woods, W. A., Fliegelman, M. J., and Chirigos, M. A.: Effect of levamisole on the *in vitro* immune response of spleen lymphocytes. *Proc. Soc. Exper. Biol. and Med.* (In Press, April 1975).

Serial No. Z01-CP-04880-04-VB

1. Viral Biology Branch
   OADVO, Division of
   Cancer Cause and Prevention
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunological and Virological Studies on Human and Animal Tumors

Previous Serial Number: SAME

Principal Investigator: Dr. Gary R. Pearson

Other Investigators: Dr. Maneth Gravell, VBB, NCI
Dr. Jean-Marie Prevost, VBB, NCI

Cooperating Units: Inside NIH
Dr. John Pearson, VBB, NCI
Dr. George Vande Woude, VBB, NCI
Dr. Stephen Davis, VBB, NCI
Dr. Dharam Ablashi, VLLB, NCI

Outside NIH:
Dr. Harvey Rabin, Litton Bionetics, Inc.
Kensington, Maryland
Dr. Donald Fine, Frederick Cancer Research Center
Frederick, Maryland
Dr. Minoru Harada, Shionogi Research Institute
Osaka, Japan

Man Years:

Total:	4.3
Professional:	1.0
Other:	3.3

Project Description

Objectives:

1. To investigate the potential usefulness of well-characterized immune serum in the treatment of animal tumors and to define the mode of action of these sera in conferring immunity to tumor transplants.

2. To study the immunological and virological aspects of *Herpesvirus saimiri* (HVS) oncogenesis in nonhuman primates as a model for Epstein-Barr virus (EBV) infection in man.

3. To collaborate with other investigators within and outside NIH on problems of mutual interest.

Methods Employed:

1. Mice were immunized with mouse sarcoma virus (MSV) or allogeneic tumor cells producing virus. At appropriate times following the immunizations, the mice were bled and the sera pooled into different groups depending on the method of immunization. The sera were heat-inactivated and then tested for antibodies directed against MSV-associated antigens by cell membrane immunofluorescence (MF). The sera were then tested both by passive transfer experiments and with *in vitro* assays for the presence of immunity factors directed against Moloney virus-induced tumors.

2. Sera from MSV-infected mice were tested for antibodies capable of mediating the destruction of cells producing Moloney virus by normal or immune lymphoid cells. Cells labeled with ^{51}chromium served as target cells in this antibody-dependent lymphocyte cytotoxicity (ADLC) assay. Five x 10^3 labeled target cells were mixed with 2.5 x 10^6 splenic lymphocytes in the presence of different dilutions of antibody-negative or positive serum. Following an 18 hour incubation period, the cytotoxicity of the serum-lymphocyte mixtures was determined by measuring the radioactivity in the cell-free supernatants. ADLC was calculated by subtracting the cytotoxicity figure of the lymphocyte in normal serum from the cytotoxicity value of lymphocytes in the corresponding dilution of immune serum.

3. Sera from HVS-infected owl monkeys were assayed for antibodies capable of mediating antibody-dependent lymphocyte cytotoxicity (ADLC) against ^{51}chromium-labeled virus-infected or uninfected owl monkey kidney (OMK) cells. Five times 10^3 viable labeled cells were incubated with 5 x 10^5 rhesus monkey peripheral blood lymphocytes, separated on Ficoll-Hypaque gradients, in the presence of normal or immune serum for 4 hours. The cytotoxic effects of the mixtures were determined by measuring the radioactivity in the cell-free supernatants following this incubation period. ADLC was calculated by subtracting the cytotoxicity value for the lymphocytes alone from the cytotoxicity figure in the presence of the test serum.

4. The specificity of the ADLC reaction in the herpesvirus system was determined by absorption of ADLC-positive sera with different numbers of antigen-positive and control cells. Absorptions were carried out for 3 hours at 37°C and for 18 hours at 4°C.

5. Sera were collected from monkeys infected with HVS or EBV at different time intervals following the initiation of an experiment. They were heat-inactivated and tested for antibodies to membrane antigens (MA), late intracellular antigens (LA) or early antigens (EA) specific for HVS or EBV

depending on the intent of the experiment. Sera from EBV-infected animals were also tested for antibodies to Epstein-Barr virus-induced nuclear antigen (EBNA). All immunofluorescence assays were performed by the indirect method using fluorescein isothiocyanate-conjugated goat anti-human gamma globulin.

6. Human sera were tested for antibodies to EBV-induced MA by the ADLC assay. Five times 10^5 human peripheral blood lymphocytes were incubated with 5 x 10^3 EBV-infected ^{51}chromium-labeled Raji cells in the presence of antibody-negative or positive human serum. Lymphocytes were separated in Ficoll-Hypaque gradients. The mixtures were incubated for 3 hours at 37°C. The cytotoxic effects of the lymphocytes in the presence of serum were determined by measuring the radioactivity in the cell-free supernatants following the incubation period. ADLC was calculated by subtracting the value for the cytotoxicity of the lymphocytes in the presence of normal serum from the cytotoxicity figure in the presence of the corresponding dilution of immune serum.

Major Findings:

1. Sera from mice immunized with allogeneic tumor cells producing MSV or from mice following tumor regression which contained high antibody titers to MSV-induced cell membrane antigens were effective in the passive transfer of immunity to transplanted virus-producing mouse tumors but not against nonproducer mouse tumors initiated with sarcoma virus genome-positive, leukemia virus genome-negative (S+L-) cells. These sera also enhanced the cytotoxic activity of normal or immune lymphoid cells for virus-produced cell lines in the ADLC test, indicating that this immune mechanism might be active *in vivo* in conferring resistance to virus-induced tumors. The temporal development of antibodies detected by the MF and ADLC assays was followed in relation to tumor progression in mice. MF titers developed to high titers following regression of MSV-induced tumors. Antibody levels remained low in mice with progressively growing tumors. The increase in MF titers in regressor mice corresponded closely on a temporal basis to the initiation of the tumor regression process. ADLC activity was only detected in the sera of regressor mice and again correlated on a temporal basis to the tumor regression process. These findings indicated that ADLC might be active in tumor immunity against MSV-induced tumors.

2. A variety of assays were used in attempts to detect a mouse sarcoma virus-specific antigen in S+L- cells provided by Dr. Robert Bassin, VLLB. Enhanced tumor growth of the S+L- cells but not of the parent uninfected 3T3 cells was noted in mice pretreated with serum from mice that had progressively growing tumors which contained specific enhancing factors for MSV-induced tumors. This finding suggested that the S+L- cells contained a weak antigen not expressed in the membranes of the parent cells. Lymphocytes from MSV-immune mice which were cytotoxic *in vitro* against S+L+ cells were tested against S+L- cells. Specific killing of S+L- cells was noted with speen cells harvested from mice 14-21 days post-infection with MSV. Spleens collected later than 21 days were cytotoxic to both the S+L- cells and to cells from the parent 3T3 line. These findings suggested that mice infected with MSV became sensitized to an antigen shared by S+L+ and S+L- cells, possibly a sarcoma-specific antigen, and also to another cellular antigen shared with the parent 3T3 cell.

3. Sera from owl monkeys infected with HVS were monitored at biweekly intervals for antibodies to HVS-associated antigens. The results supported the earlier findings which indicated that the anti-EA response related to the induction of malignant disease in this species. In capuchin monkeys, however, low to moderate levels of antibody to EA persisted in the absence of apparent lymphoma. Low to moderate levels of HVS could also be regularly rescued from the peripheral blood lymphocytes of these animals. This probably accounted for the persistence of anti-EA reactivity since it was previously established in owl monkeys that a quantitative relationship existed between the anti-EA titers and the number of infectious genomes rescued from peripheral blood lymphocytes.

4. Sera from squirrel and owl monkeys infected with *Herpesvirus saimiri* (HVS) mediated an ADLC reaction against virus-infected OMK cells. With squirrel monkey sera, antibody titers to HVS-associated cell MA determined by this reaction were approximately 10 times higher than when the titers were determined by MF. In HVS infected owl monkeys, ADLC titers in some serum samples were much higher than were the MF titers suggesting that, in some instances, multiple humoral factors were involved in mediating ADLC. Most of the high titered sera came from animals with malignant disease suggesting that antibody titers determined by this assay were related to disease progression. Attempts to detect viral antigen-antibody complexes in high titered sera by absorption experiments were not successful. HVS-associated antigens were not detected in the membranes of cells of an HVS-transformed, nonproducer marmoset cell line as determined by absorption experiments.

5. Owl monkeys and marmosets were infected with different strains of Epstein-Barr virus including the P3HR-1 strain and the high transforming B-95 strain. No malignant disease was noted in any of the monkeys although they all developed an enlargement of the spleen and lymph nodes which subsequently regressed. Monkeys infected with P3HR-1 virus produced antibodies only to VCA. These antibodies persisted for the duration of the experiment. In monkeys infected with B-95 virus, antibodies to VCA, EA and EBNA arose and persisted at low to moderate levels. In general, the antibody response to EA and EBNA occurred after the response to VCA.

6. The ADLC assay was applied to cells infected with EBV. Producer lymphoblastoid cultures, such as P3HR-1 and Ly-28, were not susceptible to ADLC probably because of the low percentage of MF-positive cells in those cultures. EBV-infected Raji cultures containing 30-40% MF-positive cells were suitable as target cells in this assay. Antibody titers to EBV-induced cell MA were approximately 10 times higher by this assay than those determined by MF.

7. Cell lines established in this laboratory and sera from a variety of sources have been provided to investigators within and outside NIH. Furthermore, this laboratory has served as one of the control facilities for determining anti-EBV titers on sera from a variety of places inside and outside NIH.

Significance to Biomedical Research and the Program of the Institute:

1. Investigations on the possible use of immunological factors for controlling tumor growth are of obvious importance. It is important to determine not only what immunological approaches might be applicable to the control or treatment of human disease but also to identify the active factors in these immunological preparations and to identify practical methods for monitoring immunotherapy procedures.

2. EB virus has been implicated as a candidate in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma in humans. Parallel studies in experimental animal systems involving herpesviruses or EBV itself contribute information on the role of herpesviruses in the induction of neoplasia and their potential for causing cancer in humans. This work also provides a useful system for developing, experimentally, control or therapy procedures potentially applicable to human diseases associated with these viruses.

Proposed Course:

This project is expected to terminate with the departure of the principal investigator.

Honors and Awards:

NONE

Publications:

Pearson, G.R.: Epstein-Barr Virus: One or a family of viruses? Proc. Int. Workshop on Human Tumors Associated with Herpesviruses. *Cancer Res.* 34: 1237-1240, 1974.

Ablashi, D.V. and Pearson, G.R.: *Herpesvirus saimiri*, a nonhuman primate model for herpesvirus associated neoplasia of man. Proc. Int. Workshop on Human Tumors Associated with Herpesviruses. *Cancer Res.* 34: 1232-1236, 1974.

Ebert, P.S. and Pearson, G.R.: Correlation of C-type virus (WF-1) production and heme synthesis in a rat fibroblastic cell line. *Proc. Soc. Exp. Biol. Med.* 145: 298-301, 1974.

Pearson, G.R., Rabin, H., Wallen, W.C., Neubauer, R.H., Orr, T.W. and Cicmanec, J.L.: Immunological and virological investigations on owl monkeys infected with *Herpesvirus saimiri* (HVS). *J. Med. Primatol.* 3: 54-67, 1974.

Neubauer, R.H., Wallen, W.C., Rabin, H., Pearson, G.R. and Ablashi, D.V.: Virological investigations of *Herpesvirus saimiri*-infected owl monkeys. *J. Med. Primatol.* 3: 27-40, 1974.

Goodheart, C.R., Armstrong, G.R., Ablashi, D.V., Pearson, G. and Orr, T.W.: Brief Communication: Concentration of oncogenic herpesviruses by methyl alcohol precipitations. *Appl. Micro.* 27: 988-990, 1974.

Pearson, G.R., Rabin, H. and Manaker, R.A.: Comparative biology of herpesvirus infections in relation to cancer. *Voprosi Virusiogii.* (In press).

Rabin, H., Pearson, G.R., Wallen, W.C., Neubauer, R.H., Cicmanec, J.L. and Orr, T.W.: Infection of capuchin monkeys (*Cebus albifrons*) with *Herpesvirus saimiri*. *J. Nat. Cancer Inst.* (In press).

Rabin, H., Neubauer, R.H., Pearson, G.R., Cicmanec, J.L., Wallen, W.C., Loeb, W. and Valerio, M.: Brief Communication: Spontaneous lymphoma associated with *Herpesvirus saimiri* in owl monkeys. *J. Nat. Cancer Inst.* (In press).

Harada, M., Pearson, G. Redmon, L., Winters, E. and Kasuga, S.: Antibody production and interaction with lymphoid cells in relation to tumor immunity in the Moloney sarcoma virus system. *J. Immunol.* (In press).

Davis, S., Redmon, L.W. and Pearson, G.R.: Enhancement of Moloney sarcoma virus-induced tumor growth by concanavalin A. *Immun. Commun.* (In press).

Prevost, J.M., Orr, T.W. and Pearson, G.R.: Augmentation of lymphocyte cytotoxicity by antibody to *Herpesvirus saimiri*-associated antigens. *Proc. Nat. Acad. Sci. USA.* (In press).

Pearson, G.R., Prevost, J.M. and Orr, T.W.: Detection of virus-associated membrane antigens with the antibody-dependent lymphocyte cytotoxicity test. Proc. Int. Workshop on Production and Purification of EBV and Associated Antigens. *Proc. Natl. Acad. Sci., USA* (In press).

Pearson, G.R. and Davis, S.: The immune response of monkeys to lymphotrophic herpesvirus antigens. Proc. Symp. on Immunological Control of Virus-Associated Tumors in Man. *Cancer Res.* (In press).

Serial No. Z01-CP-04881-01-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Host Immune Response in Relation to Cancer

Previous Serial Number: NCI-4871

Principal Investigator: Dr. Stephen Davis

Other Investigators: Dr. Charles W. Boone, VBB, NCI

Cooperating Units: Inside NIH
Dr. David H. Sachs, IB, NCI
Dr. Gene M. Shearer, IB, NCI

Outside NIH
Dr. Arnold D. Rubin, Mount Sinai Hospital
New York, New York
Dr. Michael Sela, Weizmann Institute
Rehovot, Israel

Man Years:

Total:	1.8
Professional:	1.0
Other:	0.8

Project Description

Objectives:

A. To study the immunologic mechanisms *in vivo* by which virally-induced tumors in normal and athymic mice fail or are allowed to grow.

B. To demonstrate that the genetic constitution of animals can determine their ability to respond to antigenic challenge.

C. To characterize the human leukemia cell immunologically and biochemically.

D. To study transfer factor from both mouse and human sources and its role in cell-mediated immunity.

Methods Employed:

Application of a radioisotopic foot-pad assay to measure cellular immunity; demonstration of cell surface markers on human lymphocytes by SRBC rosetting and immunoglobulin fluorescence techniques; characterization of lymphocytes with lymphocyte mitogens; preparation of dialyzable transfer factor.

Major Findings:

A. Moloney sarcoma virus (MSV) induces tumors in normal adult mice. The majority of these tumors regress spontaneously within 28 days. To date, little *in vivo* data is available concerning the cellular immune mechanisms involved in this regression phenomenon. Concanavalin A, a plant lectin, is believed to cause T-cell specific immunosuppression. Using this lectin we have shown that MSV-induced tumors show enhanced growth, suggesting T-cells contribute the major immunologic potential in this system. An isotopic mouse foot-pad assay measuring cell-mediated immunity showed that the cellular immunity to MSV antigens is short-lived (less than 17 days) and is associated with an immune suppression of T-cells. However, immune activity can return if the lymphocytes are first trypsinized, suggesting that cell-bound blocking factors are involved in suppression.

B. The antibody response to inbred strains of mice to certain antigens are controlled by genes located within the H-2 locus. However, no *in vivo* genetic data is available concerning the cellular immune response to these antigens. Using the isotopic foot-pad assay we have shown that cell-mediated immunity also is controlled by genes within the H-2 complex.

C. Using data obtained in this laboratory and in others, a scheme has been proposed for the differentiation of the human lymphoid system and its possible pathogenetic link to human lymphomas. Similarly, using plant mitogens, it appears that lymphoma cells can be distinguished from their normal counterparts by their unique ability to respond in a delayed fashion to phytohemagglutinin. A diagnostic test for human lymphomas appears feasible.

D. Studies were continued to determine the feasibility of using transfer factor to enhance tumor immunity.

Significance to Biomedical Research and the Program of the Institute:

The study of the induction and later suppression of virus-tumor immunity associated with tumor growth will help to determine those factors that can be manipulated to produce tumor rejection. Clarification of the genetic controls of immunity will lead to better understanding of the failure of immunologic responses against tumors. Characterization of human lymphoma cells may lead to ways of earlier detection and better chemoimmunotherapy.

Honors and Awards:

None

Publications:

Davis, S., Boone, C. W., Radin, R., Wade, M. J., and Kleiner, G.: Retention of significant antibody binding by surface antigens of FLA cells after fixation with formaldehyde and glutaraldehyde. *Immunol. Comm.* 3: 188-196, 1974.

Davis, S., and Boone, C. W.: *In vivo* effects of concanavalin A on the cellular immune response in BALB/c mice. *Eur. J. Immunol.* 4: 626-629, 1974.

Davis S., and Boone, C. W.: Inhibition of the cellular immune response to SV40 tumor cells in tumor-bearing and tumor-immune mice by concanavalin A. *J. Nat. Cancer Inst.* (In press).

Davis, S.: Moloney sarcoma virus-induced tumors in athymic (nude) mice. *J. Nat. Cancer Inst.* (In press).

Davis, S.: Genetic control of the murine cell-mediated immune response *in vivo*. I. H-2 linked control to the terpolymere Glu^{60}-Ala^{30}-Tyr^{10}. *Scand. J. Immunol.* (In press).

Davis, S., Redmon, L., Pearson, G. R.: Enhancement of Moloney sarcoma virus-induced tumors by concanavalin A. *Immunol. Comm.* (In press).

Davis. S.: Hypothesis: Differentiation of the human lymphoid system based on cell surface markers. *Blood*, (In press).

Schultz, E., Davis, S., and Rubin, A. D.: The nature of the leukemic cell in chronic lymphocytic leukemia. *Blood*, (In press).

Pearson, Gary R., and Davis, Stephen: The immune response of monkeys to lymphotrophic herpesvirus antigens. Proc. Symp. on Immunological Control of Virus-associated Tumors in Man, Bethesda, Maryland, *Cancer Res.* (In press).

Serial No. Z01-CP-04882-01-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Events in Tumor Virus Replication

Previous Serial Number: NCI-4893

Principal Investigator: Dr. George F. Vande Woude

Other Investigators: Dr. W. G. Robey, VBB, NCI
Dr. R. Ascione, VBB, NCI

Cooperating Units: Inside NIH
Dr. D. K. Haapala, VLLB, NCI
Dr. P. J. Fischinger, VLLB, NCI

Outside NIH

None

Man Years:

Total:	2.6
Professional:	0.7
Other:	1.9

Project Description

Objectives:

To investigate biochemical events associated with oncornavirus replication in relation to neoplastic transformation of host cells.

Methods Employed:

Oncogenic viruses are propagated in tissue culture. Focus-helper assays are used for the detection of some propagated and purified viruses. Other assays are: agarose gel diffusion for the detection of viral antigens; polyacrylamide gel electrophoresis and guanidine-agarose chromatography for the purification and identification of virus-specific proteins; *in vitro* labeling of purified viruses using radiolabeled amino acids and nucleic acids.

Major Findings:

More than 40 polypeptide components can be demonstrated in preparations from highly purified RNA tumor viruses. These range in molecular weight from several thousand to several hundred thousand daltons.

A 60,000 dalton polypeptide, p60, has been identified as a component of MSV (FeLV), the feline leukemia virus pseudotype of Moloney sarcoma virus. In the purified virus complex, this polypeptide occurs in greater concentration than either the p30 polypeptide of murine leukemia virus or the p27 polypeptide of FeLV. Monospecific antisera to the murine virus p30 peptide also react with p60. At least 2 antigenic interspecies determinants and murine virus p30 group antigenic determinants were present in p60. However, tests did not reveal the presence of the group specific determinants of FeLV p27 or of murine leukemia virus gp 71.

A comparison of 520-dimensional chromatographs of the tryptic hydrolysates of p60 and of murine virus p30 show many peptides to be common to both peptides. A limited digestion of the p60 component with either trypsin or chymotrypsin yields p30-35 and p20 peptides, both of which retain the group antigenic activity of the murine leukemia viruses and the interspecies activities. The p30 enzymatic cleavage product produced by both enzymes co-migrates with the murine p30 obtained from the MSV (FeLV) complex, suggesting that p60 is an uncleaved precursor of p30. Since murine sarcoma and murine leukemia virus information in MSV may be processed concurrently, sarcoma-specific sequences may be contained in p60.

Significance to Biomedical Research and the Program of the Institute:

The observations support the probability that mRNA of oncornaviruses may be translated as polycistronic protein products which are subsequently cleared into functional polypeptides. This raises the possibility that MSV-induced cell transformation may be related to aberrant protein cleavage due to a reiterated or faulty recombination of message.

Proposed Course:

Other pseudotypes of murine sarcoma virus, MSV, will be examined for the presence of large precursor virion proteins. Immuno-precipitates from radioisotope-labeled, infected cells will be analyzed for virus-specific proteins.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04883-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Virus-augmented Tumor Antigens. Immunity to Virus-induced Tumors

Previous Serial Number: SAME

Principal Investigator: Dr. Charles W. Boone

Other Investigators: Dr. Meera S. Paranjpe, VBB, NCI
Dr. Thomas W. Orme, VBB, NCI
Dr. Stephen Davis, VBB, NCI

Cooperating Units: Inside NIH
None

Outside NIH
Dr. Ronald W. Gillette, Meloy Laboratories, Inc.
Springfield, Virginia
Dr. Winifred G. Palmer, Litton Bionetics, Inc.
Frederick, Maryland

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To isolate "virus-augmented" tumor cell transplantation antigens, i.e., antigen fractions from tumors that have been infected with influenza virus for use in immunodiagnosis and immunotherapy in cancer in humans.

2. To develop immunodiagnostic uses for the "paired label" radioimmunoassay in humans.

3. To determine mechanisms of the cell-mediated immune response to virus-induced, chemically-induced, and spontaneous tumors in animals.

4. To evaluate various methods of immunotherapy in animal model systems.

5. To identify phenotype "markers" of cells *in vitro* that correlate with tumorigenicity *in vivo*, and to analyze these markers by biochemical and genetic means.

Methods Employed:

Biochemical procedures related to the isolation of tumor transplantation antigens from cultured cell lines infected with vesicular stomatitis virus and influenza virus. Radioimmune assay using radioiodine labeled immune and normal immunoglobulins. Tumor-graft rejection assays and delayed hypersensitivity assays against tumor antigens. Tissue culture and cinematographic procedures associated with the study of phenotypic characteristics of cells *in vitro* correlated with tumorigenicity *in vivo*.

Major Findings:

Virus-augmented tumor antigens and immunity to virus-induced tumors: A tumor-specific foot-pad reaction in mice, analogous to a tumor-specific skin test reaction in humans, against membrane fractions of tumor cells has been developed. Fractions from tissue-cultured tumor cells elicited much stronger foot-pad reactivity if the tumor cells were first infected with influenza virus. Such "virus-augmented" skin test antigens from cultured human tumors have been prepared and are awaiting clearance by the Division of Biologics before being tested in humans. Materials from 3M KCl extracts of virus-infected tumor tissue were further fractionated using differential solubility in KCl solutions. A fraction was found which induces tumor immunity approximately equivalent to a 30-fold increase in effectiveness over X-irradiated viable tumor cells as immunogen. 3M KCl was also used to isolate soluble Gross mouse leukemia virus-induced surface antigens from AKR lymphoma cells. Immunological "eclipse" in a tumor-bearing animal, that is, the specific suppression of the anti-tumor immune response in animals bearing a tumor that has reached a certain size, was clearly demonstrated for the first time *in vivo* by the increase in tumor colonies in the lung following intraveous inoculation of tumor cells into mice bearing growing tumors. "Homing" studies using ^{51}Chromium-labeled lymphoid cells in tumor-bearing animals revealed a severe depression of lymphoid cell homing to the bone marrow and spleen in tumor-bearing animals.

Criteria of oncogenesis *in vitro*: The BALB/3T3 line, used by many investigators as the normal fibroblast line to which virus and chemically transformed derivatives are compared, was itself shown to form highly malignant tumors when the BALB/3T3 cells were implanted into mice subcutaneously while still attached to their glass or plastic tissue culture substrates. The tumors that formed were malignant hemangioendotheliomas, which are tumors of vascular endothelial cells. This finding will require a major reevaluation of the criteria currently used to diagnose oncogenicity *in vitro*.

Significance to Biomedical Research and the Program of the Institute:

Virus-augmented tumor skin test antigens in purified form offer the promise of the diagnosis by a skin test of cancer in human patients or the determination of the location of the primary tumor when only a secondary metastasis is found. Virus-augmented tumor transplantation antigens also offer potential value for the immunotherapy of human tumors.

The determination and elucidation of "markers" characteristic of malignancy in tissue cultures cells from the basis for a study of the genetic basis of malignant transformation at the cellular level by somatic cell hybridization techniques.

Proposed Course:

Purification of virus-augmented tumor rejection antigens will continue until highly active preparations are available from a variety of tumor types. The "paired label" radioimmune assay will be applied to the diagnosis of soluble antigens, antibodies, and antigen-antibody complexes in the serum of patients with Burkitt's lymphoma. Investigation of the tumorigenicity of cell lines implanted subcutaneously while attached to solid substrates will be expanded to other tissue cultured cell lines, both normal and malignant. The transformation of adult connective tissue cells will also be pursued.

Honors and Awards:

None

Publications:

Gillette, Ronald W., and Boone, Charles W.: Kinetic studies of macrophages. VI: The effect of the presence of a solid tumor. *Res. J. Reticuloendothelial Soc.* 15: 126-131, 1974.

Gillette, Ronald W., and Boone Charles W.: Changes in the homing properties of labeled lymphoid cells caused by solid tumor growth. *Cellular Immunology*, 12: 363-369, 1974.

Boone, Charles W., Paranjpe, Meera, Orme, Thomas, and Gillette, Ronald: Virus-augmented tumor transplantation antigens: Evidence for a helper antigen mechanism. *Int. J. Cancer* 13: 543-551, 1974.

Paranjpe, Meera S., and Boone, Charles W.: Kinetics of the anti-tumor delayed hypersensitivity response in mice with progressively growing tumors: Stimulation followed by specific suppression. *Int. J. Cancer* 13: 179-186, 1974.

Palmer, W. G., Orme, T. W., and Boone, C. W.: Presence of a unique serum protein in mice bearing a variety of tumors. *J. Nat. Cancer Inst.* 52:279-282, 1974.

Paranjpe, M., and Boone, C. W.: Stimulated growth of syngeneic tumors at the site of an ongoing delayed hypersensitivity reaction to tuberculin in BALB/c mice. *J. Nat. Cancer Inst.* 52: 1297-1299, 1974.

Davis, S., Boone, C. W., Radin, R., Wade, M. J., and Kleiner, G.: Retention of significant antibody binding by surface antigens of feline leukemia cells (FLA) after fixation with formaldehyde and glutaraldehyde. *Immunological Communications* 3: 189-196, 1974.

Brandchaft, Phyllis B., and Boone, Charles W.: Increase in Gross (G) antigen sites on the surface of AKR virus-induced rat lymphoma cells after treatment with trypsin. *Journal of Immunology* 113: 94-102, 1974.

Orme, Thomas W., Boone, Charles W., and Roller, Peter P.: The analysis of 2-acetamido-2-deoxyaldose derivatives by gas-liquid chromatography and mass spectrometry. *Carbohydrate Res.* 37: 261-266, 1974.

Gillette, R. W., and Boone, C. W.: Effect of tumor immunity on the distribution of labeled lymphoid cells in tumor-challenged mice. *Cell. Immunology* 14: 386-383, 1974.

Davis, S., and Boone, C. W.: The *in vivo* effects of concanavalin A on the cellular immune response in BALB/c mice. Inhibition of the delayed hypersensitivity reaction to tuberculin. *Eur. J. Immunol.* 4: 626-629, 1974.

Boone, C. W.: Augmented immunogenicity of tumor cell homogentates infected with influenza virus. *Recent Results in Cancer Research* 47: 394-400, 1974.

Brandchaft, Phyllis B., and Boone, Charles W.: Extraction of Gross (G) surface antigens from AKR mouse lymphoma cells with 3M potassium chloride. *J. Nat. Cancer Inst.* 53: 1079-1083, 1974.

Paranjpe, Meera S., and Boone, Charles W.: Specific depression of the anti-tumor cellular immune response with autologous tumor homogenate. *Cancer Research*, (In press).

Boone, Charles W.: Malignant hemangioendotheliomas produced by the subcutaneous inoculation of Balb/3T3 cells attached to glass beads. *Science*, (In press).

Serial No. Z01-CP-04884-02-VB

1. Viral Biology Branch
OADVO, Division of Cancer
Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Events of *Herpesvirus saimiri* (HVS) Replication *In vitro*.

Previous Serial Number: SAME

Principal Investigator: Dr. Josephine Simonds

Other Investigators: Dr. George F. Vande Woude, VBB, NCI
Dr. Bettie Graham, VBB, NCI

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.4
Other:	0.1

Project Description

Objectives:

To separate and characterize components of *Herpesvirus saimiri* (HVS) virions and study the replication of this virus *in vitro* by biochemical techniques.

Methods Employed:

Herpesvirus saimiri is grown in quantity by methods developed in this laboratory. The virus is labeled isotopically with ^{3}H- or ^{14}C-thymidine. DNA is extracted from the virions and banded by equilibrium centrifugation in cesium chloride density gradients.

Major Findings:

The DNA of purified HVS could be resolved into 2 components by equilibrium centrifugation in CsCl, one of which had the expected density of 1.727 gm/cm^3 and the other a density of 1.709 gm/cm^3. The DNA from virus that had 3 sequential plaque purification steps yielded a similar pattern of density

distribution. The higher density DNA co-sedimented with herpes simplex virus type 1 DNA. The lighter component was definitely heavier than the owl monkey kidney host cell DNA.

If HVS was treated with DNase prior to disruption of the virions and extraction of their DNA, 4 DNA components were distinguished on equilibrium centrifugation in CsCl: 1.727; 1.718; 1.712; and 1.706 gm/cm^3.

Significance to Biomedical Research and the Program of the Institute:

Herpesvirus saimiri is of particular interest because it induces T-cell lymphoid neoplasia in certain non-human primates. The detection of multiple DNA species in this virus is unusual and their significance with respect to the transforming properties of the virus requires investigation. The development of methodology for large scale production of this virus will now facilitate characterization of virion polypeptide components and the investigation, biochemically, of the events associated with the replication of this virus *in vitro*.

Proposed Course:

Further characterization of the multiple species of DNA of HVS using restriction enzymes will be done in collaboration with Carel Mulder from the Cold Spring Harbor Laboratory and with Bernard Fleckenstein from the Universitat Erlangen-Nuremberg. Some consideration will be given to mapping of the HVS genome.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04886-01-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetics of Herpesvirus DNAs

Previous Serial Number: NONE

Principal Investigator: Dr. Bettie J. Graham

Other Investigators: Dr. G. F. Vande Woude, VBB, NCI
Dr. W. G. Robey, VBB, NCI

Cooperating Units: Inside NIH
Dr. D. Haapala, VLLB, NCI

Outside NIH

None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description

Objectives:

To determine differences between the DNAs of parental herpesviruses and the DNAs of the viral progeny produced in dual infections with other viruses in cultured cells.

To determine the state of the viral genome(s) in the infected cells.

Methods Employed:

This project was only recently initiated. Two available virus-cell systems will be examined: a herpes simplex virus type 1 (HSV-1) superinfection of a mouse bone marrow cell line carrying Rauscher mouse leukemia virus, JLSV-9 (RLV), and a HSV-1 superinfection of the P3HR-1 lymphoblastoid human cell line carrying Epstein-Barr virus (EBV). DNA-DNA hybridization methods will

be applied. DNA fragments will be generated by restriction enzymes for use in hybridization and to determine differences in the cleavage products between viruses.

Major Findings:

The isolation of purified high molecular weight viral DNAs was begun. Modifications of nucleic acid hybridization methods were evaluated for application to this project.

Significance to Biomedical Research and the Program of the Institute:

This project is expected to provide specific information on the possibility for genetic modification of viruses in dually infected host cells which may bear upon the oncogenic potential of these agents.

Proposed Course:

Studies are in progress to compare fragments of different herpesvirus DNAs generated by restriction enzymes. The degree of homology will be determined between the DNAs of HSV-1 prior to and after its modification by passage through Rauscher leukemia virus-infected cells. Attempts will be made to determine whether HSV-1 DNA becomes integrated into host cell DNA after infection of P3HR-1 lymphoblasts.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04887-01-VB

1. Viral Biology Branch
   OADVO, Division of
   Cancer Cause and Prevention
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Molecular Biology of Oncogenic DNA Viruses

Previous Serial Number: NONE

Principal Investigator: Dr. Maneth Gravell

Other Investigators: None

Cooperating Units: Inside NIH
Dr. Gary Pearson, VBB, NCI

Outside NIH
None

Man Years:

Total:	1.8
Professional:	0.8
Other:	1.0

Project Description

Objectives:

To use virological and biochemical techniques to investigate the relationship of herpesviruses to the etiology of human and animal cancers.

Methods Employed:

1. Herpes simplex viruses, types I and II (HSV-I, HSV-II) and *Herpesvirus saimiri* (HVS) have been propagated in cultured cells and purified by differential and buoyant density centrifugation techniques. Herpesvirus DNA has been labeled by culturing virus in the presence of radioactive precursors or by use of an *in vitro* enzymatic technique employing *E. coli* DNA polymerase. Radiolabeled DNA is used to probe tumor or *in vitro* transformed cells for herpesvirus-specific genetic information by molecular hybridization techniques. Hybridization of DNA probes with herpesvirus sequences is detected by hydroxyapatite column chromatography or by enzymatic methods employing specific nucleases.

2. Indirect immunofluorescence techniques were used to detect Epstein-Barr virus-specific antigens (viral capsid antigen, early antigen and nuclear antigen) in lymphoblastoid cell lines or in cells of various tumors.

Major Findings:

Since this work was only recently started, major emphasis has been placed on developing methodology needed to detect herpesvirus genetic information by renaturation kinetics or other molecular hybridization techniques. Much of this developmental work has been completed and studies are being initiated to probe tumor or *in vitro* transformed cells for herpesvirus genetic information. Methodology has also been developed to assay herpesvirus DNA for infectivity or transforming ability *in vitro*. Of the three methods compared (DEAE-dextran, polybrene, and calcium phosphate), infectivity of herpesvirus DNA has been greatest when the calcium phosphate method has been used.

We have established a lymphoblastoid cell line from a tumor mass of an American Burkitt's lymphoma patient. This lymphoblastoid cell line, designated NAB, contains the EBV genome. Two strains of this cell line have been isolated. One strain (NAB-2) spontaneously produces EBV, while the other strain (NAB-1) is a non-producer of EBV capsid and early antigens. NAB-1 cells can be induced to produce EBV early antigens by treatment with halogenated pyrimidine.

Significance to Biomedical Research and the Program of the Institute:

Although herpesvirus has been associated with human malignancies, including Burkitt's lymphoma, nasopharyngeal carcinoma and cervical carcinoma, they have not been established as unequivocal causes of these diseases. The complete EBV genome is present in tumor cells of African Burkitt's lymphoma and nasopharyngeal carcinoma; however, at most, a fragment of the HSV-II genome is present in cervical carcinoma tumor cells. Herpesvirus genetic information would be expected to be present in cells of these tumors, unless its presence is unrelated to the transforming event. A more thorough understanding of the mechanisms involved in regulation of EBV genetic information in transformed cells is necessary. In addition, more sensitive techniques are needed to probe cervical carcinomas for HSV-II antigens and genetic information. Future research will be focused in these general areas.

Proposed Course:

Emphasis will be placed on developing sensitive molecular hybridization techniques to detect herpesvirus genetic information in tumor and *in vitro* transformed cells. When these techniques have been perfected, they will be applied to obtain a better understanding of the expression and regulation of EBV and HVS genetic information in transformed cells. If satisfactory progress is made in the above studies and time permits, cervical carcinoma tissue and cells transformed *in vitro* by HSV-II will be probed for HSV-II information. The use of bacterial restriction enzymes are contemplated in this work.

Honors and Awards:

None

Publications:

Levine, P.H. and Gravell, M.: The viral etiology of leukemia. *Modern Pediatrics* 16: 136-164, 1974.

Twardzik, D.R., Aaslestad, H.G., Ablashi, D.V., Tureckova, M.I., Gravell, M. and Levine, P.H.: Comparison of DNA polymerase activities from an American Burkitt's lymphoma cell line and EBV producer and non-producer cells. 2nd International Symposium on Oncogenesis and Herpesviruses. (In press).

Goldberg, R.J. and Gravell, M.: A search for HSV-2 molecular markers in cervical carcinoma. Proceedings of the Symposium on "Immunological Control of Virus-Associated Tumors in Man." *Cancer Res.* (In press).

Serial No. Z01-CP-04890-04-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Purification and Characterization of Virus Associated Tumor Transplantation Antigens

Previous Serial Number: SAME

Principal Investigator: Dr. Thomas W. Orme

Other Investigators: Dr. Charles W. Boone, VBB, NCI
Dr. Peter Roller, CMT, NCI

Cooperating Units: Inside NIH:

None

Outside NIH
Ms. Billie McCaw, Litton Bionetics, Inc.
Frederick, Maryland
Ms. Faye Austin, Meloy Laboratories, Inc.
Springfield, Virginia
Dr. Ann Boyd, Litton Bionetics, Inc.
Frederick, Maryland
Dr. Winifred Palmer, Litton Bionetics, Inc.
Frederick, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To isolate and characterize tumor transplantation antigens using the following virus-tumor-mouse systems: (a) SV40-transformed BALB 3T3 fibrosarcoma in BALB/c mice; (b) murine mammary tumor virus (MuMTV)-induced mammary adenocarcinoma in C3H mice; (c) Moloney murine leukemia virus (MuLV)-

induced ascites lymphoma in A/He mice; and (d) Herpes simplex type 2 (Savage strain)-induced fibrosarcoma in BALB/c mice.

2. To define the role of virus-induced antigens or virus-modified host cell antigens in tumor transplant rejection.

3. To develop a reliable alternative to the tumor rejection assay for measuring tumor transplantation antigen activity.

4. To develop protocols for the use of transplantation antigens in immunotherapy and immunodiagnosis.

5. To demonstrate carbohydrate in tumor transplantation antigens and to determine its biological significance.

Methods Employed:

The following techniques were applied: tumor transplant rejection assay and delayed hypersensitivity tests to measure tumor antigens; carbohydrate analysis by radio-gas chromatography; fractionation of extracted tumor antigens by gel permeation chromatography, ion exchange chromatography, isoelectric focusing, affinity chromatography and preparative differential centrifugation; detection of virus-associated antigens by indirect immunofluorescence techniques.

Major Findings:

1. A rapid mouse delayed hypersensitivty (DH) test performed with tumor-bearing animals was developed to screen normal and transformed cells syngeneic to BALB/c mouse tissue for antigens which cross-react with SV40-transformed BALB 3T3 cells. It was shown that seven days after subcutaneous injection of 1 X 10 viable SV40-transformed BALB 3T3 cells into 6-8 week old BALB/c males, the mice acquire cell-mediated sensitivity to specific tumor cells inoculated into their footpads. This sensitivity is suppressed as the tumor load increases. The assay detected cross-reactivity in three tumor cell lines previously considered to be unrelated except for their common BALB/c mouse heritage: a) SV40-transformed embryonic endothelial cells (BALB 3T3); b) herpes simplex type 2-transformed embryonic mouse skin cells; and, c) LSQ-3, a tissue culture line derived from a methylcholanthrene-induced squamous cell carcinoma. Syngeneic primary embryo cultures and adult fibroblasts showed no cross-reactivity, nor did ten other syngeneic tumor lines arising by spontaneous, chemical or viral transformation.

2. Tumor cells extracted with 3M KCl yield soluble antigens which react in the DH test described above. In general, cells which cross-react in the tumor rejection (TR) assay yield soluble antigens which cross-react in the DH test; the converse has not been thoroughly tested. Isoelectric focusing has led to some enrichment of the antigens involved, but fractionation studies have been hampered by the loss of biological activity. Two important facts about the 3M KCl extraction procedure which were discovered earlier, but neglected, have gained new significance. The extraction of tumor antigens from cells is

incomplete, and the soluble extracts do not protect mice in the TR assay. The extracted cells were reexamined and shown to induce full TR activity as well as strong DH activity. These cells are now considered a good killed cell vaccine and can reasonably be substituted for X-irradiated cells or extracts in skin tests and in immunotherapy. A working hypothesis which encompasses the accumulated information is that 3M KCl extraction partially removes an incomplete tumor transplantation antigen detectable by the DH test, but not by the TR assay and completely removes inhibitory substances (or blocking factors) which usually make killed tumor cells poor immunogens.

3. BALB/c embryonic mouse skin cells transformed by UV-irradiated herpes virus type 2 (HSV-2) (Savage strain) cause fibrosarcomas when injected subcutaneously into normal adult BALB/c mice. The tumors are transplantable and possess a tumor transplantation antigen which can be demonstrated by immunizing normal mice with X-irradiated tumor cells. The transformed cells produce HSV-2 associated antigens detected with antisera having specificities for HSV-2 virions. Serum from tumor-bearing mice neutralizes HSV-2, yet there is no evidence of virus formation in the transformed cells. Tumor bearer sera also reacts with tumor cells in a complex fashion involving both nuclear and cytoplasmic antigens. The pattern of reactivity is dependent on tumor size.

Significance to Biomedical Research and the Program of the Institute:

The experiments suggest a method for preparing reagents potentially useful in the immunodiagnosis and therapy of human cancer. The work is contributing to an understanding of the host and virus factors controlling expression of tumor transplantation antigens.

Proposed Course:

A comprehensive program to compare the specificities of the DH and TR assays will be undertaken to determine what antigens are being measured by these tests.

Honors and Awards:

None

Publications:

Orme, Thomas W., Boone, Charles W., and Roller, Peter P.: The analysis of 2-acetamido-2-deoxyaldose derivatives by gas-liquid chromatography and mass spectrometry. *Carbohydrate Research* 37: 261-266, 1974.

Palmer, Winifred G., Orme, Thomas W., and Boone, Charles W.: Presence of a unique serum protein in mice bearing a variety of tumors. *J. Nat. Cancer Inst.* 52: 279-282, 1974.

Boone, Charles W., Paranjpe, Meera, Orme, Thomas W., and Gillette, Ronald: Virus augmented tumor transplantation antigen: Evidence for a helper antigen mechanism. *Int. J. of Cancer* 13: 543-551, 1974.

Boyd, Ann L., Orme, Thomas W., and Boone, Charles W.: HSV-2 transformation in a murine system. *2nd International Symposium on Oncogenesis and Herpesviruses*, Nuremberg, 1974 (In press).

Serial No. Z01-CP-04892-04-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of *In Vitro* Behavior and Mechanisms of Immunogenicity of Neoplastic Cells

Previous Serial Number: SAME

Principal Investigator: Dr. Meera Paranjpe

Other Investigators: Dr. Charles W. Boone, VBB, NCI

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To correlate the saturation density, anchorage dependence, and surface structures, such as agglutinating sites, with the characteristics of malignancy of transformed human and murine cell lines cultured on adherent and nonadherent substrates.

2. To further chemically characterize the phenomenon that inoculated tumor homogenate induces a specific depression of the anti-tumor cellular immune response.

Methods Employed:

1. Time-lapse cinematography of malignant and normal cell lines cultured on various adherent and nonadherent substrates.

2. A radioisotopic foot-pad assay for the study of the *in vivo* cellular immune response in the mouse.

3. A ^{51}Cr release assay for studies on cellular immunity *in vitro*.

Major Findings:

1. Teflon discs can be used as a selective substrate for the growth of malignant cell lines. Neoplastic cultured cell lines of human and murine origin proliferated on Teflon substrates whereas similarly seeded, normal, nontumorigenic cells were unable to do so. Six murine and three human cell lines were used in this study. Teflon was also a very useful substrate for determining the anchorage dependence of cultured cell lines.

2. The optimum concentration of dib-cAMP (dibutyrl cyclic adenosine monophosphate) was established that would restore saturation densities of cultured neoplastic cells to those of their normal counterparts. Anchorage dependence was completely restored in the SV40 virus-transformed 3T3 cell line and in the TACT line (tumorigenic adult mouse connective tissue, passage 10) by addition of dib-cAMP to the culture medium and was partially restored in more highly tumorigenic mouse cell lines such as T238 (TD_{50} = 1 X 10^6), M3 (TD_{50} = 1 X 10^6) and M3T (TD_{50} = 1 X 10^3).

3. A homogenate of an SV40-transformed fibrosarcoma of BALB/c mice (E_4 tumor) injected intraperitoneally into E_4 tumor-immune syngeneic mice specifically depressed their cell-mediated immune response to autologous tumor cells as measured by a radioisotopic foot-pad assay. When the tumor homogenate was extracted with 3M KCl, soluble and insoluble active fractions were identified. The serum of E_4 tumor immune mice, but not of normal mice, that had been injected 24 hours previously with E_4 tumor homogenate suppressed anti-tumor cell immunity *in vitro* as measured by the release of ^{51}Cr from labeled E_4 tumor cells incubated with spleen cells from tumor-immune animals.

Proposed Course:

The exact point in the cell cycle will be determined where blockage occurs in cells unable to grow on Teflon. The effect of dib-cAMP on microtubule assembly and Con A agglutinating sites of malignant cells will be studied.

Significance to Biomedical Research and the Program of the Institute:

1. The selective growth on Teflon of neoplastic cells of human and murine origin may provide a simple technique for obtaining pure tumor cell cultures from some primary human tumors.

2. Time-lapse microcinematography permits a serial analysis of the changes in the relationship of malignant cells toward each other. It is now accepted that these changes are caused, at least partially, by altered cell surface

characteristics. Studies involving cell-substrate adhesivity, anchorage dependence, and surface structures, such as agglutinating sites, of neoplastic cells are important in elucidation of the changes associated with malignancy.

Honors and Awards:

None

Publications:

Paranjpe, M. S., and Boone, C. W.: Kinetics of the anti-tumor delayed hypersensitivity response in mice with progressively growing tumors, stimulation followed by specific suppression. *Int. J. of Cancer* 13: 179-186, 1974.

Boone, C. W., Paranjpe, M., Orme, T. W., and Gillette, R.: Virus-augmented tumor transplantation antigens. Evidence for a helper antigen mechanism. *Int. J. of Cancer* 13: 543-551, 1974.

Paranjpe, M. S., and Boone, C. W.: Stimulated growth of syngeneic tumors at the site of an ongoing delayed hypersensitivity reaction to tuberculin in BALB/c mice. *J. Nat. Cancer Inst.* 52: 1297-1299, 1974.

Paranjpe, Meera S., and Boone, Charles W.: Specific depression of the anti-tumor cellular immune response with autologous tumor homogenate. *Cancer Research*, (In press).

Paranjpe, M. S., and Boone, C. W.: Intermittent sphering of virus-transformed and other neoplastic cells observed by time lapse cinematography. *Exptl. Cell Res.*, (In press).

Serial No. Z01-CP-04895-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical and Immunological Characterization of Oncornavirus and Herpesvirus Virions and Specific Polypeptides

Previous Serial Number: SAME

Principal Investigator: Dr. W. Gerald Robey

Other Investigators: Dr. G. F. Vande Woude, VBB, NCI
Dr. B. J. Graham, VBB, NCI

Cooperating Units: Inside NIH
Dr. G. R. Pearson, VBB, NCI

Outside NIH

None

Man Years:

Total:	2.2
Professional:	0.6
Other:	1.6

Project Description

Objectives:

To investigate mechanisms of host cell modulation of virus genetic expression.

Methods Employed:

Herpesviruses were propagated in cell cultures and quantitated by plaque assay and electron microscopy. Immunoflourescence methods were applied to determine the presence and the degree of herpesvirus infection. Clones were established from cultures dually infected with different herpesviruses. Routine methods were followed in tests for virus neutralization, for virus stability and for virus infectivity for animals.

Major Findings:

The P3HR-1 cell line of lymphoblasts originating from a Burkitt's lymphoma produces Epstein-Barr virus (EBV) in small amounts. When this cell line was superinfected with herpes simplex virus type 1 (HSV-1), little or no difference in the growth rate of the cells was observed. However, the chronically-infected cultures were found to continuously release HSV-1 at titers of 10^6 to 10^7 PFU per ml of culture fluid. After several serial subcultures, the differences in virus yield that related to the multiplicity of infection disappeared, and about 80 percent of the cells were positive for HSV-1 antigens by immunofluorescence whereas the number of cells demonstrating the presence of EBV capsid antigens remained stable at 10 percent. The HSV-1 produced by the dually-infected lymphoblasts is specifically neutralized by antisera; and the plaque morphology, neuropathology in mice, and the buoyant density determined by cesium chloride density gradient centrifugation remained unchanged from that exhibited by the parent virus stock. These cultures are unique in exhibiting no cytopathology despite continuous maturation of HSV-1, suggesting that the host cell control of EBV expression may be affecting the cell-virus relationship with HSV-1.

Significance to Biomedical Research and the Program of the Institute:

This virus-cell system permits the study of host cell control over virus genome expression in human cells whereby biochemical events associated with the modulation of virus activity may be determined.

Proposed Course:

The P3HR-1 cells producing HSV-1 will be synchronized to determine whether HSV production parallels EBV expression during the cell cycle. Cloned cell lines will be developed to determine whether HSV-producing and HSV-nonproducing cultures can be obtained for investigations on the intracellular state of the herpesvirus genome.

Honors and Awards:

None

Publications:

Robey, W.G. Further characterization of an antibody stimulating factor from bovine thymus. *N.Y. Acad. Sci.* (In press).

Serial No. Z01-CP-04896-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Analysis of Gene Controlled Events in Neoplastic Transformation

Previous Serial Number: SAME

Principal Investigator: Dr. Stephen J. O'Brien

Other Investigators: Dr. Charles W. Boone, VBB, NCI

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. Examination of feline leukemia virus (FeLV) and murine leukemia virus (MuLV) to investigate the regulation of infection, production and assembly of oncornavirus and virus-associated cell surface antigen (CSA). This includes: (a) study of the kinetics of biosynthesis of FeLV and FeLV CSA; (b) assessment of the effect of streptonigrin on several aspects of FeLV infection and production; (c) characterization of CSA with respect to virion components; (d) characterization of the degree of cross-reaction of FeLV-CSA and MuLV-CSA; (e) characterization of the regulation of expression of FeLV and CSA in cat x mouse cell hybrids.

2. Examination of the character, extent and regulation of cellular structural gene dysfunction in transformed cells. The analysis includes: (a) examination of 20 randomly selected isoenzyme gene products for gene dysfunction in transformed tissue culture lines; (b) examination of 25 major soluble proteins in the same lines; (c) examination of surface

components of the same lines. The analysis is being performed on chemically and virally transformed cell lines; (d) genetic analysis of the regulation of aberrant gene products and their relationship or synteny to the transformed phenotype.

3. Genetic analysis of tumorigenicity in culture, including: (a) segregation analysis of tumorigenicity and chromosomal segregation in somatic cell hybrids; (b) genetic complementation matrix of tumorigenicity in a number of independently transformed lines.

Methods Employed:

The following techniques are used: (a) radio-iodine labeling of antisera for detection of cell surface antigens; (b) lactoperoxidase labeling of cell surfaces with radio-iodine; (c) starch gel and polyacrylamide gel electrophoresis (PAGE), and isoelectric focusing; (d) *in vitro* radioisotope assay of RNA-dependent DNA polymerase as a viral marker; (e) somatic cell hybridization and various cell culture procedures; (f) chemical mutagenesis of cells and selective procedures for acquisition of selectable transformed cell lines; (g) cytological chromosome banding procedures.

Major Findings:

The kinetics of FeLV virus biosynthesis was studied in the presence of inhibitors of transcription (actinomycin D), mRNA processing (cordycepin), and translation (cycloheximide). Differential response to cordycepin compared to actinomycin indicate that a minimum of two RNA species with different lifetimes and processing requirements participate in virion assembly. The average turnover rate of virus-associated cell surface antigens (CSA) was estimated as approximately five hours. The shortest lived mRNA required for virus production turns over in two to four hours while the rate limiting mRNA necessary for CSA synthesis persists for ten hours. The lack of correspondence of kinetics of biosynthesis of virus particles and cell surface antigens, which are largely virus structural proteins, reflects the participation of a number of RNA species in the production of these viral components in infected cells. We have determined that at least 50% of FeLV CSA is identical with FeLV p27, the major FeLV structural protein which carries the gs-1 and gs-3 antigenic determinants.

The character and extent of cross-reaction between cell surface antigens on feline and murine cell lines infected with oncornavirus was estimated with respect to three types of antigenicity: humoral, cell mediated, and tumor protection. Using two broadly-reactive antisera and four antisera monospecific to virion proteins, the extent of CSA cross-reaction was quantitated between the two species. The pattern of cross-reaction between cat and mouse viral-associated CSA was complex and dependent upon the specificity of the test antiserum. In general there was significant cross-reaction with broadly

reactive serum against either viral-induced tumors or purified virus as well as cross-reaction with monospecific antisera prepared against the viral proteins p27, p30 and gp69-71. Cross-reaction of cell-mediated immunity between the cat and mouse systems was demonstrated at about 10 percent above control levels in mice with a radioisotopic foot-pad assay. Tumor protection experiments in mice failed to detect cross-reaction between the two species with vaccines of FeLV infected cells.

Somatic cell hybrids between FeLV infected cat cells and mouse cells preferentially segregated feline chromosomes. To date, over twenty hybrids examined fail to express either FeLV or FeLV-CSA. Experiments designed to determine if the activity is a mouse-mediated suppression of FeLV or a segregation of a necessary feline chromosome product are in progress.

Streptonigrin, an anti-tumor drug, was titrated for its effects on FeLV infection, production, and CSA synthesis. Effects on all aspects of viral and CSA production except infection are apparently non-specific.

Twenty methylcholanthrene-transformed cell lines were examined by PAGE of cell sonicates for dysfunction of major soluble proteins compared to normal fibroblast lines. Of 24 bands which were monitored in densitometric scans, only one peak varied consistently; and in only two of the twenty lines was this observed. Of 8 isozyme systems examined in these same cell lines there was considerable variation both quantitatively and qualitatively in a number of the MCA lines examined. The variations were not random and followed definite constraints in their type of variation. The isozyme aberrations are being used as cell markers in fusion experiments designed to examine the genetic regulation of gene dysfunction and malignancy following neoplastic transformation.

Significance to Biomedical Research and the Program of the Institute:

The analysis of the metabolism of leukosis virus infection and its relationship to antigen assembly provides important information about the course of cell modification as the result of tumor virus infection and expression. The understanding of the virus and host genetic machinery active in a malignant cell is necessary for any meaningful attempt to correct or destroy cancerous tissue. The resolution of surface antigens on the virus-infected cell surface and the comparative immunogenicity of cell surface antigens are of immense importance in the evaluation of immunotherapeutic procedures for tumor cell protection and removal. The analysis of host gene dysfunction will be the first attempt to provide a thorough study of the biochemical character of aberrant host gene action following neoplastic transformation. The parasexual genetic analysis of gene dysfunction and tumorigenicity is an important approach to the understanding of genetic regulation of cell division in normal and malignant mammalian cells.

Proposed Course:

A continuing study of gene action and the genetic regulation of transformation is in progress. The application of findings as diagnostic tools as well as for a means to fuller understanding of gene derepression in cancer is anticipated.

Honors and Awards:

None

Publications:

O'Brien, S. J., and Shimada, Y.: The α-glycerophosphate cycle in Drosophila melanogaster. IV. Metabolic ultrastructural and adoptive consequences of αGpdh^{-1} null mutations. *J. Cell Biology* 63: 864-882, 1974.

Serial No. Z01-CP-04897-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Interaction of Herpesvirus and Oncornavirus in Mixed *in vitro* Infections.

Previous Serial Number: SAME

Principal Investigator: Dr. Angelo P. Andrese

Other Investigators: Dr. Bettie Graham, VBB, NCI
Dr. George F. Vande Woude, VBB, NCI

Cooperating Units:

Inside NIH
Dr. Daniel Haapala, VLLB, NCI
Dr. Berge Hampar, VCB, NCI

Outside NIH
None

Man Years:

Total:	2.3
Professional:	1.2
Other:	1.1

Project Description

Objectives:

To investigate interactions *in vitro* between herpes simplex virus type-1 (HSV-1) and Rauscher leukemia virus (RLV) in mouse bone marrow derived cells (JLSV9) chronically infected with RLV (JLSV9-RLV).

Methods Employed:

HSV-1 was grown in primate VERO cells, and subsequently plaque purified 5 times by standard techniques. Virus neutralization, immunofluorescence, and electron microscopy procedures were applied.

Major Findings:

Two strains of plaque-purified HSV-1 were used, the C173 and the MAL strains. When either strain was serially passaged in JLSV9 cells chronically infected with RLV (JLSV9-RLV), a HSV population emerged characterized by the presence of a double envelope surrounding the virion nucleocapsid. In addition, the morphology of the lesions produced on chicken embryo fibroblast cultures, as well as on other cells, was changed from the round cell form exhibited by the parent HSV to a syncytial form. These changes did not occur when the same parental strains of HSV were passaged through JLSV9 cells not infected with RLV, nor when the viruses were passaged in VERO cells. Virus from 5 of 7 plaque selections of HSV predominantly produced syncytia after passage through the RLV-infected culture but not after passage through uninfected JLSV9 or VERO cells. Reversion to round cell plaque morphology did not occur when syncytium-producing virus was repeatedly passaged through VERO cells. The emergent strain exhibits properties similar to those of other syncytium-producing strains of HSV in its unrestricted ability to grow in chicken embryo fibroblasts, its thermolability, and its slightly lighter density than the parent strain. It is antigenically indistinguishable from the parent strain.

Significance to Biomedical Research and the Program of the Institute:

Herpesviruses as well as Type C RNA viruses are implicated in the cause of certain animal tumors and both groups of agents are suspected to contribute to oncogenesis in humans. Whether herpesvirus-associated neoplasia involves interaction with a Type C virus genome pre-existing in the host cells is unknown. This project indicates that genetic modification or selective processes may occur in cells dually infected by members of these two groups of viruses. Whether this interaction may influence neoplastic response by infected cells remains to be determined.

Proposed Course:

Other cells, including human cells, will be studied to determine whether the biological and biochemical changes observed in the virus-cell system described will be reproduced. Tests will be made to determine whether the oncogenic potential of the HSV emergent from RLV-infected cells has been affected. Attempts will be made to obtain knowledge of the mechanisms involved in the emergence of virus with altered characteristics.

Honors and Awards:

None

Publications:

Andrese, A.P., Walling, M.J., and Vande Woude, G.F. Herpes simplex virus superinfection of mouse cells chronically infected with Rauscher leukemia virus. *Proc. Electron Microscopy Soc. of Amer.* 32:106-107, 1974.

Andrese, A.P., Vande Woude, G.F., and Walling, M.J. Ultrastructure study of herpes simplex virus infection of mouse cells chronically producing Rauscher leukemia virus. *J. Nat. Cancer Inst.* 53: 1169-1173, 1974.

Smida, J., Smidova, V., Andrese, A.P., and Vande Woude, G.F. Search for oncogenicity to type-C particles released from mammalian cell lines transformed by avian oncornaviruses. *Neoplasma* 21: 609, 1974.

Serial No. Z01-CP-04899-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Virus and Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Control Through External Inhibitors of Intracellular Biochemical Changes in Tumor Cells and/or Induced by Viral Infection

Previous Serial Number: SAME

Principal Investigator: Dr. Takis S. Papas

Other Investigators: Dr. Michael A. Chirigos
Dr. Wilna A. Woods
Dr. John W. Pearson

Cooperating Units: Inside NIH:
Dr. Samuel H. Wilson, LB, NCI
Dr. Franklin H. Portugal, VCB, NCI
Dr. Robert J. Crouch, LMG, NICHHD

Outside NIH:
Dr. Nicholas L. Petrakis, University of California, San Francisco, California
Dr. J. G. Chirikjian, Georgetown School of Medicine, Washington, DC
Dr. R. D. Irwin, Georgetown School of Medicine, Washington, DC
Dr. Richard Axel, Institute for Cancer Research, Columbia University, New York, New York
Dr. Donald C. Fish, Frederick Cancer Research Center, Frederick, Maryland

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

1. To investigate the biochemical role of RNA-directed DNA polymerase of RNA tumor virus by using specific inhibitors of the enzyme. The immediate aims are (a) to develop potent inhibitors of reverse transcriptase, (b) to study the specificity of the inhibitors by comparison with other normal cellular polymerase, (c) to understand the molecular events on inhibition and correlate such information with antiviral and antitumor activity.

2. To investigate the mechanism of action of viral DNA polymerase from different sources, determine differences or similarities in biochemical properties, and compare viral transcriptase with normal cellular polymerase.

3. To study interaction of 70S AMV RNA with purified AMV DNA polymerase.

Methods Employed:

Avian myeloblastosis virus (AMV) obtained from chicken plasma was concentrated and purified by published standard procedures. Other Type C particles were isolated by isopycnic banding on sucrose gradients.

AMV, RLV, and RSV DNA polymerase were highly purified. The purification steps involved detergent treatment, column chromatography (DEAE, phosphocellulose), isoelectric focusing.

Cellular polymerase(s) obtained from cultured cells were fractionated through several column chromatographic procedures.

Viral 70S RNA is being extracted under ribonuclease-free conditions using sucrose gradients. Adenine residues of 70S AMV RNA were chemically modified with chloroacetaldehyde.

Major Findings:

1. Protection of avian myeloblastosis virus (AMV) DNA polymerase by substrate against heat inactivation: Purified DNA polymerase from AMV was exposed to thermal inactivation in the presence or absence of various compounds required for maximal activity. A synergistic protective effect was noted when all the components were present. The enzyme showed a distinct preference for primed templates, especially for its native 70S RNA. Heat inactivation studies also suggested that the active sites for RNase H and that for the polymerase were not identical.

2. Affinity chromatography of viral DNA polymerases on pyran-sepharose: Pyran covalently linked to cyanogen bromide-activated sepharose has been shown to be an effective affinity matrix for several viral DNA polymerases. Differential salt elution of viral versus cellular polymerases as well as substrate elution has verified the affinity nature of the matrix. Unlike other affinity systems

described, pyran-sepharose is totally resistant to nuclease digestion and was found to be stable at 4° for several months. DNA polymerases isolated from several viruses, disrupted by detergents were recovered in good yield Iodination of the purified protein and fractionation of SDS-gel electrophoresis showed essentially a pure protein resulting from a single pass from the column. These results suggest pyran-sepharose to be an effective affinity column which is potentially adaptable as part of a general purification for viral DNA polymerases.

3. Subunits of the DNA polymerase of avian myeloblastosis virus (AMV): DNA polymerase from avian myeloblastosis virus (AMV) contains two structurally distinct forms (α,$\alpha\beta$). Both enzyme forms demonstrate polymerase and ribonuclease H activities (Grandgenett, D. P., et al. PNAS, USA, 70, 230, 1973). The minor species (α) can be generated from the major species ($\alpha\beta$) by chromatography on phosphocellulose, DNA cellulose, glycerol gradient and isoelectric focusing. The isoelectric point (ip) for α and $\alpha\beta$ were 7.0 and 5.5 respectively. The conversion of $\alpha\beta$ to α was dependent on glycerol concentration with total conversion occurring at a concentration of 50%. The α subunit has a higher Km value for synthetic template-primer than $\alpha\beta$ indicating that the α subunit plays a role in the binding of the enzyme to the polynucleotide substrate. The activities of both α and $\alpha\beta$ subunits were inhibited with antibody raised against $\alpha\beta$ indicating a common origin of these enzyme forms.

4. The inhibition of oncornavirus DNA polymerase by anthracycline compounds: Anthracycline compounds have been reported to possess antitumor and antiviral activity. Experiments were undertaken to determine a possible mechanism of antiviral action. Five anthracycline compounds, adriamycin, rubidazone, 13-dihydro-daunomycin, daunomycinone, and daunorubicin, were tested for their ability to inhibit oncornavirus DNA polymerase activity. All compounds were found to be potent inhibitors of DNA polymerase purified from avian myeloblastosis virus and murine leukemia virus (Rauscher). Inhibition was primarily due to the tetracyclic planar ring structure (daunomycinone) common to all of these compounds. The inhibition could be overcome by addition of template. Addition of enzyme had no effect on the degree of inhibition. Inhibition was template specific for the templates tested: activated DNA, synthetic hybrids, poly $rA \cdot dT_{12-18}$, and poly $rC \cdot dG_{12-18}$, and the synthetic copolymer, poly(dA-dT). Absorption spectrum studies, as well as the studies stated above, indicated that the drugs bind to the template and not to the enzymes. The drugs were also tested against various bacterial and mammalian polymerases. The comparative inhibitory effects attained on the latter polymerases indicated a more specific inhibitory activity of the anthracycline compounds for the viral polymerases.

Significance to Biomedical Research and the Program of the Institute:

It is clear that the formation, the maintenance, and the expression of provirus are the central features of the life cycle of RNA tumor viruses. Many questions related to these aspects are unsettled. Elucidation of the nature of the enzymes and other factors involved in each step of viral replication and of their mechanism of action will be essential to gain an

understanding of the process of oncogenesis induced both by exogenous and endogenous viruses. Inhibitors which block each of these steps will be useful in studying the regulation of gene expression at various stages of viral replication. An inhibitor binding to the polymerase molecules or to nucleic acid templates could act in the regulation of cell functions. Such an inhibitor could be used in the development of chemotherapy for controlling diseases directed by nucleic acids (i.e. viral infections and probably cancer).

Proposed Course:

1. To continue the search for natural and synthetic compounds that possess antipolymerase activity.

2. To study the mechanism of inhibition of viral and cellular polymerases.

3. To study the mechanism of transcription of 70S AMV RNA and purified viral or cytosine residue of 70S RNA and the polymerase. To fractionate specific oligonucleotide fragments derived from modified 70S RNA which may be specifically inhibitory for the enzyme.

Honors and Awards:

None

Publications:

Papas, T. S., Pry, T. W., and Chirigos, M. A.: Inhibition of RNA-dependent DNA polymerase of avian myeloblastosis virus by pyran copolymer. *Proc. Nat. Acad. Sci.* USA, 71: 367-370, 1974.

Chirikjian, J. G. and Papas, T. S.: Inhibition of AMV DNA polymerase by polyriboadenylic acid containing ε-adenosine residues. *Biochem. Biophys. Res. Comm.* 59: 489-495, 1974.

Papas, T. S., Chirikjian, J. G., Pry, T. W., Massicot, J., Irwin, R. D., and Chirigos, M. A.: Effect of chemically-modified 70S RNA from avian myeloblastosis virus (AMV) upon the activity of AMV DNA polymerase. *J. Virology* 14: 1108-1114, 1974.

Papas, T. S., Chirigos, M. A., and Chirikjian, J. G.: Protection of avian myeloblastosis virus (AMV) DNA polymerase by substrates against heat inactivation. *Nucleic Acids Research* 1: 1399-1410, 1974.

Schafer, M. P., Chirigos, M. A., and Papas, T. S.: Inhibition of Rauscher leukemia virus and avian myeloblastosis virus DNA polymerase by tilorone and its analogs. *Cancer Chem. Repts.* 58: 821-827, 1974.

Chirigos, M. A. and Papas, T. S.: Immunological and chemotherapeutic prevention and control of oncogenic viruses. *Advances in Pharmacology and Chemotherapy* 12: 1-35, 1975.

Chirikjian, J. G., Rye, L., and Papas, T. S.: Affinity chromatography of viral DNA polymerases on pyran-sepharose. *Proc. Nat. Acad. Sci.* (In Press, March 1975).

Papas, T. S., Chirikjian, J. G., Woods, W. A., and Chirigos, M. A.: Active site probes and the selective inhibition of RNA tumor viral DNA polymerase. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Woods, W. A., Papas, T. S., and Chirigos, M. A.: The effect of levamisole on *in vitro* parameters of the immune response. Fogarty International Center Proceedings, No. 28, U. S. Government Printing Office, Washington, DC (In Press, 1975).

Serial No. Z01-CP-04902-03-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cell Free Biosynthesis of Oncornavirus-specific Polypeptides

Previous Serial Number: SAME

Principal Investigator: Dr. Richard Ascione

Other Investigators: Dr. W. G. Robey, VBB, NCI
Dr. G. F. Vande Woude, VBB, NCI

Cooperating Units: Inside NIH
Dr. D. Haapala, VLLB, NCI

Outside NIH
None

Man Years:

Total:	2.4
Professional:	1.9
Other:	0.5

Project Description

Objectives:

To determine the number and the molecular characteristics of viral specific proteins encoded in the genetic information of oncogenic viruses.

Methods Employed:

1. The isolation and the subcellular fractionation of protein synthesizing components are necessary, i.e., polyribosomes, initiation and elongation factors, transfer RNA, and aminoacylsynthetases.

2. Milligram quantities of messenger RNA are prepared from a variety of sources: avian myeloblastosis virus (AMV)-infected cells; simian sarcoma virus (SSV)-infected NC37 cells; Moloney sarcoma virus (MSV)- and feline leukemia virus (FeLV)-infected cat cells (P521); and MSV and murine leukemia virus (MuLV)-infected mouse cells (3B11).

3. Preparations of messenger RNA from infected cell polyribosomes and purified virus were isolated by utilizing a combination of sedimentation velocity and equilibrium density gradients. The isolated RNA was further purified by oligo-dT cellulose or poly-U-sepharose column chromatography; and the messenger RNA was utilized in a cell-free protein synthesizing system derived from uninfected calf fetal organs (brain, kidney and liver). After suitable incubation, the radioisotopically prepared proteins were isolated by immunoprecipitation with the appropriate antiserum to specific viral components. This protein was further analyzed by various methods and identified as to its specific viral components. Analysis consisted of polyacrylamide gel electrophoresis and Ouchterlony techniques combined with radioisotopic autography to identify oncornaviral specific components.

Major Findings:

RNA isolated directly from oncornaviruses can be used for the cell free synthesis of oncornavirus proteins. The RNA must be of the high (>35S) molecular weight type and not contaminated by the smaller inhibitory RNA fragments generally sedimenting less than 30S. The virus production and purification procedures devised by laboratory circumvent contamination by low molecular weight fragments by using high salt concentrations and polyethylene glycol precipitation of the large molecular weight species and by further purifying the RNA by the use of poly-U-sepharose which selects out the poly A-containing sequences of the intact molecules. Such RNA species isolated from AMV have been able to effect the synthesis of proteins which co-migrate in polyacrylamide gels with the authentic viral components of AMV.

To date, however, direct translation of oncornavirus RNA has only been possible with AMV-RNA due to the short supply of RNA from other oncornaviruses. Consequently, other methods were selected for isolating high molecular weight RNA from oncornavirus-infected cells, particularly from cells infected with non-avian oncornaviruses. For this purpose, techniques were perfected which isolate intact polyribosomes from infected cells for use as a source of virus specific messages. Although such preparations are contaminated with host cellular messages, the viral proteins synthesized from a heterologous system could be resolved by immunoprecipitation with an antiserum prepared against specific virus components or even disrupted whole virions. Since infected cell polyribosomes are more readily available in larger quantities than are purified virions, this method may prove to be applicable for the resolution of virus-specific components, especially when virus is in limited supply. Preliminary experiments with this system enabled the isolation of messenger RNA which stimulates the translation of polypeptide products. The specific nature and the functions of these proteins remain to be resolved.

An outgrowth of the preparation of polyribosomes from feline leukemia virus/murine sarcoma virus-infected cells, has been the characterization of the different species of polyribosomes, particularly with emphasis on enzymatic activity associated with membrane bound polyribosomes. The highest specific activity of reverse transcriptase enzyme was associated with polyribosomes which band isopycnically in 57% sucrose. However, releasing membrane-bound

polyribosomes with detergent removes 90% of the enzymatic activity as well as the activity that co-sediments with ultralarge polyribosomes, ≥500S, corresponding to an aggregate of 40-50 ribosome units or larger.

Significance to Biomedical Research and the Program at the Institute:

These studies should unambiguously resolve the question which proteins are specified by the oncornavirus genome, thereby yielding specific information as to the molecular characteristics of many of the different types of oncornavirus proteins. Presumably, such studies could also yield information regarding the relationship of the oncornavirus to its host, and whether or not the sarcoma-leukemia viruses possess common structural and functional similarities. Furthermore, such studies may yield information as to which proteins are directly involved in the tumorigenic process and which virus proteins are essential to the oncogenic properties of tumor viruses.

Proposed Course:

The above studies are being expanded to include primate sarcoma and leukemia messenger RNA and their translational expression. Such cell-free characterization may reveal significant features common to all oncornavirus proteins, as well as identify which polypeptides and genomic segments are essential for oncogenic expression.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04903-01-VB

1. Viral Biology Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Local Adoptive Transfer of Anti-tumor Cellular Immune Response in Syngeneic, Allogeneic and Xenogeneic Recipients

Previous Serial Number: NONE

Principal Investigator: Dr. Noritoshi Takeichi

Other Investigators: Dr. Charles W. Boone, VBB, NCI

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To define further the nature of the foot-pad assay for anti-tumor immunity by histological and immunological means.

2. To study the adoptive transfer of the anti-tumor cellular immune reaction in mice to allogeneic mice and xenogeneic rats with specific immune T-lymphocytes.

3. To determine the possibility of transferring human cellular immunity against skin test antigens and tumor antigens to rats and mice as the basis of a bioassay for anti-tumor immunity in humans.

Methods Employed:

Biochemical procedures related to the isolation of tumor transplantation

antigens from cultured cell lines infected with vesicular stomatitis virus and influenza virus. Radioimmune assay using radioiodine labeled immune and normal immunoglobulins. Tumor-graft rejection assays and delayed hypersensitivity assays against tumor antigens. Tissue culture and cinematographic *in vitro* correlated with tumorigenicity *in vivo*.

Major Findings:

1. Several nonspecific and specific components of the anti-tumor cellular immune reactions in foot-pads were identified: (a) edema and vascular changes secondary to the mechanical trauma of the inoculum; (b) progressive growth of inoculated tumor cells; (c) the specific anti-tumor cellular reactions mediated by T-lymphocytes; (d) delayed hypersensitivity reactions; additional factors in allogeneic and xenogeneic recipients were: (e) nonspecific acute inflammatory reactions to allogeneic or xenogeneic inoculum; (f) a host versus graft reaction; (g) a graft versus host reaction. Methods for eliminating nonspecific factors (a), (b), (e), and (f), which disturb the specific foot-pad reaction in syngeneic, allogeneic and xenogeneic recipients were developed. Thymus-independent cells of host origin were found to be necessary for the manifestation of the anti-tumor foot-pad reaction. A close correlation was found between immune responses detected by the foot-pad assay and those detected by transplantation techniques.

Significance to Biomedical Research and the Program of the Institute:

The radioisotopic foot-pad assay is rapid, quantitative, specific and can be applied not only in allogeneic recipients but also in xenogeneic recipients with adoptive transfer of specific immune T-lymphocytes. Therefore, this assay may be useful in humans in determining the immune status of the cancer patient.

Proposed Course:

The immunologic nature of the foot-pad reaction in xenogeneic system (rat to mouse) will be analyzed. The eclipse phenomenon, i.e., the specific suppression of anti-tumor immunity by a growing tumor will be studied by adoptive transfer of lymphocytes obtained from rats with growing tumor to xenogeneic mice. In the next year, attempts will be made to demonstrate the adoptive transfer of human cellular immunity to skin test antigens and tumor antigens to rats and mice, using the radioisotopic foot-pad assay.

Honors and Awards:

None

Publications:

None

SUMMARY REPORT

b. VIRAL CARCINOGENESIS BRANCH

July 1, 1974 - June 30, 1975

Introduction

It is not generally understood that ascertainment of the viral cause(s) of cancer and of achieving prevention of cancer are highly interdependent objectives. All virus diseases are multifactoral in origin, requiring permissiveness on the part of a variety of both viral and host factors. This conceptual approach to etiology of virus disease unfortunately is not sufficiently understood by many cancer investigators, yet it is indispensable to all successful efforts to prevent or to modify viral diseases. The natural virus specific immune responses resulting from spontaneous infections or from the use of type specific viral vaccines have been clearly and repeatedly shown to prevent or to greatly modify many naturally occurring diseases. Thus the causative roles of viral agents in many serious diseases of animals as well as of man were solidly established at the same time that these diseases were prevented by virus specific vaccines. The causative role of poliovirus and of many other viruses responsible for categorical diseases could be made no more certain than was achieved by their prevention with the use of virus specific vaccines; obviously the key word in this context is "virus-specificity."

During the past two years, the research program of the Viral Carcinogenesis Branch has been focused on the concept that the specific causative etiology of many cancers in feral and experimental animals, and by inference also in man, can best be established by prevention of naturally occurring cancers with the use of virus specific vaccines and/or by clearly identified and isolated natural genetic inhibitors.

Although occasional exogenous oncornaviruses have been found to transmit horizontally, the postulate that endogenous oncornavirus genomes are major inherited causes of cancer has gained much new support in FY 1975 from the discovery by Virus Cancer Program supported scientists of the genetically transmitted xenotropic RNA tumor viruses in many animals from mice to primates. The substantiating basis and data for this view and the successful research approach it has engendered is represented in an impressive body of knowledge which in FY 1975 clearly established endogenous oncornaviruses as inborn causes of cancer in inbred mice, wild mice, cats, and almost as certainly in domestic animals (cattle and swine) and a variety of primates of which gibbon apes and baboons are prime examples, this despite the fact that certain oncornaviruses also have the ability to transmit horizontally or vertically. Data substantiating these statements will be documented not only in this report but also in reports from other Branches of Viral Oncology and from the Segment Contract Programs.

Although extensive searches for a resident human oncornavirus have been negative to date, there can be little doubt that man harbors a genome similar to that found in virtually all other animals which have been adequately studied (see listing of known oncornaviruses, Tables 1a, 1b, and 1c, appended.) Since the scientists in the Viral Carcinogenesis Branch are collaborating extensively with colleagues scattered throughout the Virus Cancer Program, with scientists in other Branches in Viral Oncology, and at least 10 different research contracts within the Solid Tumor Segment (STS) program, precise credits to those responsible for new findings and significant advances may be difficult to pinpoint; thus the joint findings of VCB and STS scientists will be discussed together. Individual project and contract reports from Viral Oncology and Segment Chairmen should, however, clarify such issues. The chief aim in this summary is to present a coherent picture of the current state of the art concerning the role of oncornaviruses in the etiology of cancer and the contributions to this picture made by VCB and STS scientists and then of course to describe the work currently in progress to determine the causes and the possible modes of prevention of cancer.

Objectives in the Viral Carcinogenesis Branch and Solid Tumor Segment

The major aims of the collaborative VCB and STS programs can perhaps best be put in the terms of questions that form the basis for the various Tasks, each one currently representing a major area of inquiry.

Task I. Are endogenous as well as exogenous RNA tumor viruses (types C and B) oncogenic in natural hosts? Detailed data given below makes it clear that endogenous ecotropic RNA tumor viruses are oncogenic in natural species. Current evidence also supports oncogenic activity on the part of the xenotropic viruses; however, data on these slowly acting viruses are less extensive at this time because of the longer incubation (well over 12 to 24 months) required before cancer occurs in those strains which have only the xenotropic virus.

Task II. How do cellular genes determine natural and induced cancer in inbred mice? Can the gene products be used to control cancer not only in inbred and outbred mice, but in other higher species, and eventually man? The answers to this complex question are being intensively pursued in VCB and STS collaborative programs under the leadership of Drs. Aaronson, Stephenson, Levy, Meier, Lilly, Rowe and others. Certain gene complexes (Fv-1 and MuLV-1) and their products which serve as regulators of oncornavirus expression and replication in inbred mice, and more importantly as genetic factors controlling their spontaneous tumors as well, have not only been identified but their loci are now well known and some gene products have already been tested for potency (Tennant and Brown.)

Task III. Are immune responses involved in the prevention of natural cancer and, more importantly, will virus specific RNA virus vaccines prevent spontaneous cancers in those classes of animals wherein the causative

viruses have been identified? The answer to this question promises to be in the affirmative, as will be described below. Preliminary studies by VCB and STS contract scientists, using combined killed and live vaccines in young inbred mice, suggest that oncornavirus replication can be significantly suppressed by vaccine induced antiviral immunity and thus natural cancers of mice can be expected to be reduced if not wholly prevented.

Task IV. Do environmental factors (carcinogenic chemicals, radiation, DNA viruses) cause cancer by acting in concert with endogenous RNA tumor viruses? There is considerable new as well as old data suggesting that this is true; thus cancer in this context would be the result of "cocarcinogenesis". As will be described later, animal cells infected naturally or artificially in vitro with replicating ecotropic or xenotropic RNA viruses are much more readily transformed by carcinogenic chemicals and "oncogenic" DNA viruses. This important finding by VCB and STS scientists working in concert had an additional payoff in that it led to the development of highly sensitive, rapidly performed in vitro test systems capable of detecting carcinogenic agents belonging to a wide variety of differing categories.

Task V. What will answers to the above four questions mean as far as human cancer is concerned? The answer to this fifth question depends on the development of effective long-term protection against cancer by oncornavirus specific vaccines developed in suitable animal model systems, but final success in vaccine modification of human cancer will depend entirely on successful isolation of bona fide human oncornaviruses. All current evidence suggests that this should eventually be achieved since type C and B RNA tumor viruses have been visualized in human as well as primate placentas and tumors.

Accomplishments During FY 1974

Task I. Oncogenicity of Endogenous Oncornaviruses.

The discovery early in 1974 by Drs. Levy, Pincus, Aaronson, Todaro, Arnstein, Hartley, and many others (see Table 1a) of xenotropic endogenous viruses in all strains of laboratory mice and subsequently in baboons and other primates led to new studies designed to elucidate the involvement in cancer not only of the xenotropic viruses, but that of endogenous ecotropic viruses as well.

Although established RNA tumor viruses are by definition oncogenic, it cannot be assumed that all the endogenous ecotropic or xenotropic RNA viruses are equally oncogenic. Prior to and during FY 1975, oncogenic behavior on the part of both N and B ecotropic viruses was substantiated when such viruses were tested by inoculation of appropriately susceptible mice; however, long incubation periods prior to tumor induction were usually required. However, the question remained: were these viruses also clearly responsible for spontaneous tumors in their natural hosts?

A variety of experimental studies confirmed that they were responsible. In our report last year we cited the studies of Meier, Taylor, Cherry, and Huebner; Lilly, Duran-Reynals, and Rowe; Aaronson and Stephenson; Hartley; and Gardner and Henderson which established quantitative correlations of murine type C RNA virus expressions, the p30 viral protein in spleens and/or infectious virus in tails of mice, with subsequent incidences of spontaneous leukemias, lymphomas and other tumors. Although the studies implicated genetic control mechanisms were surely responsible, significant immune response reactions were also shown to be involved in susceptibility or protection.

Studies by Meier and Heiniger (published) in FY 1974 and subsequent studies by Huebner, Meier and Heiniger (unpublished) revealed in the HRS system that the tails of homozygous hr/hr mice which develop 50-60% lymphoma in 12 months in every case contained nearly 5-fold more infectious type C virus than did the lymphoma free congenic litter mates hr/+ and +/+. (See Fig. 1, appended.)

Repeated experiments left little doubt but that the single hairless gene was somehow responsible for the differences in both lymphoma and virus expressions. However, that differences in immunological responses were also involved was determined by treatment of hr/hr, hr/+ and +/+ mice with Imuran (the antibody suppressant azathioprine),which resulted in 60% lymphoma rates in mice from all three congenic lines in a 12 month period. Heiniger also showed that hr/hr mice were relatively deficient in their general immune responses as compared with the hr/+ and +/+ which no doubt in some part contributed to the high leukemia incidence in hr/hr mice. These observations confirm again the efficacy of natural immune responses in preventing spontaneous tumors in natural hosts.

Other observations showing close correlations between RNA tumor virus expressions in early life and cancers later in life include Gardner et al. (USC) studies of weanling wild mouse spleen and tail virus expressions which were quantitatively related to the subsequent development of leukemias during the first 12 to 18 months of life. It has become obvious from these and numerous other observations that the titers of virus in the early life of inbred mice is invariably the main determinant of high and low incidences of cancer later in life. Several exceptions to this rule, DBA/2, RF/AKR and SWR/AKR crossbred mice have high virus expressions but low spontaneous lymphoma rates in the first 12 months of life. In each instance early treatment with immune suppressants such as Imuran intramuscularly (IM), 1% 3-methylcholanthrene on the skin, or low doses of nitroso compounds during late pregnancy results in nearly 100% lymphoma within the first year of life. These and other observations to be discussed later demonstrate that there are two powerful host controls of cancer, one at the cellular level wherein virus and tumor expressions are controlled by cell gene products and the other at the level of the whole organism wherein the host's immune response mechanisms, including humoral and cell-mediate immunity are decisive in determining the levels of the RNA tumor virus burden and the development of cancer (see Task III.)

Task II. How do cellular genes control cancer?

Studies of cancer in inbred mice in the early 1900's by Slye and particularly C. C. Little and others established gene segregation as a critical determinant of the incidence of cancer. Later other investigations at the Jackson Laboratory and elsewhere (Snell, Russell, Heston and Lilly) using mendelian techniques of cross-breeding identified numerous histocompatibility and other genes that influenced both the types and the incidences of cancer. In recent years new technologies including virological, antigenic and immunological assays, and molecular probes for viral genes in cells have been added to the mendelian analyses of gene segregation, thus making it possible to determine both the presence of specific virus structural genes and the controlling action of host cell genes which regulate expressions of the RNA tumor viruses including those of cancer. Consequently, Lilly, Meier, Rowe, Aaronson, Stephenson, and their associates have now established specific locus sites for certain type C RNA virus structural genomes and also for genes which regulate these well-defined inherited cancer inducing genomes; thus in the inbred mouse the viral oncogene theory now appears in large part to be rather well established.

Recently Tennant et al. basing his experiments on Lilly's and Hartley's and Rowe's demonstration that Fv-1^n and Fv-1^b genes reciprocally suppress the replication of the N and B tropic viruses found in mouse cells attempted to extract active virus inhibitors from N and B type cells grown in culture. They apparently had considerable success because crude extracts of N and B cells significantly reduced titers of infectious N and B virus in their respective cells, the induction in virus titers measuring as much as 80% of the original titers. This exciting observation encouraged Drs. Aaronson, Stephenson and Portugal in VCB to mount a targeted program aimed at more precise definition of host gene controls of the RNA tumor virus expressions using available modern viral, antigenic and molecular probes in efforts to isolate natural gene products which inhibit virus and tumor activity in vivo as well as in vitro. Although the ecotropic viruses are well understood and the tumors they induce more easily studied, the xenotropic viruses are more universal, hence Aaronson and his group have mounted large scale crossbreeding experiments utilizing various inbred strains of mice known to have only xenotropic virus. Aaronson's and Levy's group have identified 5 strains of such mice--NZB, 129/J, C57L/J, SWR/J and NIH Swiss. The NZB mice have high xenotropic virus expressions, while the others are generally low expressions, the 129/J being the lowest; in fact virus infectious for xenotropic virus susceptible cells has not been isolated despite the fact that 129/J does express the p70 protein of type C viruses. This antigen has been uniquely identified by Boyse et al. and Lerner et al. as the protein product of the classical murine gene G_{1X+}; thus providing additional evidence that gene subunits of type C RNA virus are indeed structural genes of natural species.

Prevention of cancer in wild mice (Lake Casitas strain) by genetic control of type C RNA viruses. Gardner and his associates (USC) with Meier (JAX), Rowe (NIAID) and Huebner (VCB) have shown that the natural virus expressions of amphitropic and ecotropic viruses of the Lake Casitas wild mice in Los Angeles which are naturally responsible for significant amounts of lymphoma and motor neuron paralysis (about 20% in the first year of life) are virtually eliminated in F_1 crosses and backcrosses to the C57BL/10/Sn mouse, a mouse which is homozygous dominant for suppression of ecotropic and amphitropic RNA tumor virus expressions, thus p30 infectious virus, tumors and paralysis are suppressed (see Table 2.) This study shows that genetic engineering of this type not only eliminates virus and tumor expressions but also points to the possible value of genetic counseling in high cancer risk groups if and when similar information provided by epidemiological, antigenic and molecular probes becomes available in human cancer.

Role of xenotropic viruses in cancer. The universal presence of xenotropic RNA virus genomes in the cells of mice, cats, rats, hamsters, swine, baboons and other primates has several important implications. Since there is no conceivable mode for epigenetic transmission in natural hosts, these findings (see Table 1a) appear to establish in these classes of animals the virogene part of the viral oncogene hypothesis proposed 5 years ago; this hypothesis was the basis of a theory which stated that the genomes of RNA tumor viruses should be universally present as inherited structural operons in vertebrates. Precisely because the xenotropic viruses cannot infect their natural host cells, it will be more difficult to determine their role in the cancers of their natural hosts.

It was predicted and is already clear that those strains of mice known to have only xenotropic viruses would have low incidences of cancer, particularly in the first year of life, since most have low levels of spontaneous virus expression and at the same time, in most instances, show high levels of natural neutralizing antibody in their sera to the xenotropic viruses. The differing amounts of xenotropic virus expression, high in NZB and low in NIH Swiss and SWR, C57L and 129/J, have provided opportunities for carrying out mendelian studies of the genes coding for their structural and regulating mechanisms. The latter appear to be generally different from those regulating the ecotropic viruses. Crossing the two extremes, NZB X NIH Swiss or NZB X 129/J should result in segregations of high and low virus titers and high and low tumor responses (Aaronson and Stephenson, and Levy.)

Are the xenotropic viruses oncogenic? This seems likely because all of the known strains of mice having only xenotropic virus develop various spontaneous cancers at quite significant levels during the second year of life. Thus, studies by VCB and STS contract scientists (Kelloff, Huebner, Peters, Meier and others) reveal the following: (1) C57L mice are devoid of significant p30 expression in spleens and thymuses until 400 days of age; after 400 days, many of the mice become positive for p30 and between 450

and 600 days of age about 25% of the mice develop p30 antigen positive reticulum cell sarcomas. (2) SWR/J and NIH Swiss mice having p30 expressions in adulthood develop a wide variety of cancers, mostly in the second year. (3) NZB mice which have high levels of xenotropic virus expressions develop a lupus-like autoimmune disease,also develop lymphomas and sarcomas spontaneously, however, only at low rates after the first year.

The NZB mouse system deserves special study because it produces large amounts of endogenous xenotropic virus early in life and at the same time correspondingly large amounts of immune globulins which form antigen-antibody complexes that interfere with normal functions of the kidney and bone marrow, thus simulating the lupus syndrome in man.

Azathioprine immunosuppression of NZB mice during early life leads to early lethal leukemias, lymphomas and sarcomas, a fact that demonstrates not only the importance of immune responses in the control of the cancer but also rather strongly suggests that the large amounts of the xenotropic virus present in the leukemic tissues may be the actuating cause of the leukemias.

Task III. Natural and vaccine-induced immune responses in control of endogenous virus replication and spontaneous and induced cancers.

One of the more conclusive methods for proving the etiologic role of a virus in disease is to prevent or modify virus disease with the use of effective type-specific viral vaccines. Success in similar endeavors with RNA tumor viruses may provide a method for prevention of tumors as well as for conclusively establishing postulated RNA tumor viruses as specific causes.

Since, with some notable exceptions in highly permissive animal systems, most spontaneous cancers in natural species generally occur later rather than early in life, and since effective virus-specific neutralizing and cell-mediated immune responses can be produced early in life to endogenous virus by live attenuated and formalin-killed virus-specific vaccines, prevention of some spontaneous cancers by such vaccines now seems feasible. As mentioned earlier, effective vaccine prevention of cancer-inducing laboratory strains of well-established leukemia and sarcoma viruses has already been achieved.

Evidence supporting these statements is in part published and in part still in press or in preparation. Hence, I will cite from available sources the documentation which my associates and I believe justifies attempts to explore in depth the possibilities for viral vaccine prevention of spontaneous cancers, of cancers induced or accelerated by chemical carcinogens, and possibly also of those induced by DNA tumor viruses.

Recent experience gained during vaccine experiments which resulted in specific induction of viral-neutralizing antibodies in mice and rats should furnish useful background for future efforts to prevent and modify cancer in higher animals and man. Our reasons for selecting mice (inbred, outbred,

wild) and rats and hamsters are based on the large amount of information available in these animals concerning their natural cancer incidences, virus expressions, immune responses, and, in inbred mice, well-defined genes which regulate RNA tumor viruses and tumor expressions.

Prevention and/or moderation of cancer by natural antibodies and by immune responses induced by specific RNA virus vaccines. Virtually all viral vaccines which are successful in preventing diseases in humans have been developed and produced in tissues or cells of experimental animals and tested for protective potency and safety in experimental animals before use in humans. This is not only a humane imperative but is also, scientifically speaking, the soundest procedure for discovering and developing the most effective products prior to human trials. Thus, in 1974, VCB scientists in collaboration with colleagues in a number of Viral Cancer Program contracts, became interested in immune responses and their effects not only on spontaneous and induced lymphomas, but on other pathogenic manifestations of type C RNA viruses. For instance, the lupus-like autoimmune disease in NZB mice appears to be due to exaggerated immune responses to DNA, RNA and proteins of fully expressed xenotropic MuLV virogenes. The resulting immune complexes produce malfunctions in kidneys and bone marrow. The recent findings that both lymphoma and paralysis induced by endogenous wild mouse viruses could be prevented with specific antiviral neutralizing antisera produced in mice or guinea pigs led to a systematic study of specific vaccine modification and prevention of not only cancer but other diseases in which expressions of ecotropic and xenotropic type C viruses are involved.

The importance of knowing which immunotypes of type C RNA tumor viruses are involved. In order to hope to modify endogenous RNA virus-induced diseases (including cancer) it is essential to know as much as possible about the natural behavior and immunology of the viruses involved. Fortunately, within the last year it has been possible to characterize the biologic and molecular properties and the immunologic specificities of many of the endogenous and exogenous viruses shown in Table 1. This information plus detailed information concerning the natural histories of many type C viruses and of the tumors with which they are associated now provides a rational basis for strategic attempts at vaccine prevention of cancer.

Vaccine experiments. Working in concert with STS contract groups, VCB scientists have succeeded in producing type-specific type C RNA viral neutralizing antibodies at an early age to endogenous viruses in inbred and outbred laboratory mice and rats (Huebner, Lane, Gilden, Kelloff, Peters, Donahoe.) The neutralizing antibodies were specifically accompanied by cell-mediated antibodies.

Formalin-inactivated banded type C RNA virus vaccines produced according to specifications by Flow Laboratories, Inc. and Electronucleonics, Inc. (both funded by Dr. Gruber's Resources Program) were given to weanling BALB/c, SWR/J, C57BL/Cum, NIH Swiss, wild mice, and Fischer rats; the vaccines were made from AKR, GLV, RLV, RADLV, SLV, RALV, wild mouse and

xenotropic AT124 viruses. Except for wild mouse virus vaccine given to apparently tolerant Lake Casitas wild mice, all produced potent neutralizing antibodies which were type specific. All the antisera were produced in NIH Swiss mice, since this strain has no ecotropic virus and expresses its endogenous virus (a xenotropic virus) at very low levels, and consequently produces relatively little natural antibody to its endogenous virus during early life.

The immune response results shown in Table 3 on four different viral vaccines represent the optimum degree of specificity provided by extremely potent formalin-inactivated preparations. Less potent vaccines of the same types as those shown in the table required more inoculations, gave lower type-specific responses and frequently significant amounts of cross reactivity, the latter most likely representing nonspecific precipitation rather than specific inactivation of virus. Although there is good evidence for virus-specific cell-mediated antibody responses to several of these vaccines, the formalin-killed vaccines have had, with one exception, little or no effect on tumors induced subcutaneously by 3-methylcholanthrene.

Live attenuated viral "vaccines" induce protection against 3-methylcholanthrene-induced tumors. Early experiments revealed that specific complement-fixing and neutralizing antibodies to RADLV could be produced in C57BL mice by inoculation of RADLV itself or the MSV(RADLV) pseudotype sarcoma virus. Earlier experiments using a variety of MSV pseudotypes revealed high levels of the type-specific neutralizing antibodies in inoculated mice particularly in those wherein the MSV-induced sarcomas regressed. In Table 4 a representative cross reaction neutralization test illustrates the specificity of such reactions.

Use of RADLV in low dosage as a live virus "vaccine" in 3 experiments in C57BL/6 mice resulted in significant reductions in the incidence of subcutaneous and intramuscular fibrosarcomas induced by 3-methylcholanthrene. A fourth experiment in which the immunologically heterotypic Graffi leukemia virus was used in low dosage did not provide protection against 3MC-induced sarcomas; the fact that infection with Graffi virus was confirmed suggests that immune responses specifically to RADLV, the natural endogenous virus of C57BL mice, provided the type specific protection observed, a premise that was confirmed by neutralization tests on the sera of the mice when tested in 1974 (Capps, Trimmer and Huebner.)

A prediction of the specific viral vaccine approach postulates that it is likely to be far easier to prevent virus-induced leukemia and other cancers than to attempt therapy after the disease has been initiated. Thus while we have preliminary evidence that live attenuated MuLV's (RADLV) can produce early antiviral immunity and protection versus chemically induced tumors, good evidence that wholly inactivated vaccines will do so must still be established.

Use of both live and attenuated vaccines. Reduction of type specific virus replication in F_1's of SWR females X AKR males was observed when the maternal SWR mice were vaccinated 4 times (at 14-day intervals) with banded formalin-inactivated Gross leukemia virus, followed by injection of F_1's at 10 days of age with a maximum dose of the Gross pseudotype of Moloney sarcoma virus [MSV(GLV)]. Neutralizing antibodies to GLV were observed in the SWR females 10 to 14 days after the fourth intraperitoneal inoculation with the GLV banded vaccine. While neutralizing antibody levels in the sera of the F_1 mice (taken at 10 days) were low in titer, all of the 10-day-old mice were completely resistant to a maximum dose (10^4) of MSV(GLV) virus, while unvaccinated cohorts died by 10-12 days with large sarcomas.

Results of XC tests for infectious virus in 2% tail extracts taken from F_1's at 40 to 60 days of age are shown in Table 5. Although all unvaccinated controls revealed $>10^4$ viral content, the vaccinated mice had much lower amounts of infectious virus. This experiment is still in progress and possible vaccine effects on the development of subsequent leukemia or other cancers must still be determined; however, significant numbers of spontaneous leukemias should occur in controls within 18 months, thus providing critical information on possible immunoprophylaxis by this type of immunoprocedure.

Task IV: Endogenous RNA tumor viruses as co-determinants of cell transformation and tumors; neoplastic reactions usually attributed solely to treatment with DNA tumor viruses and/or environmental chemical carcinogens.

In vitro malignant transformation. Ongoing collaborative studies between VCB and MAI scientists provided further evidence that added exogenous as well as endogenous type C RNA virus represent critical virogenetic determinants of transformation of rat and mouse cells in vitro (Huebner, Lane, Hill, Capps, Freeman, Price, Igel and others.)

In a paper in preparation, Price et al. describe specific RLV antibody protection against malignant transformation by 3-methylcholanthrene in a well established rat cell culture chronically infected with Rauscher leukemia virus. Not only was transformation prevented but the RLV in the chronically infected cells was reduced to almost undetectable levels. RLV infected but antibody untreated cultures were readily transformed by the cocarcinogens; the use of antisera to an unrelated oncornavirus failed to protect.

As in numerous previous reports, the control rat cells which were not chronically infected with RLV did not undergo any transformation. Several repetitions of this experiment established quite conclusively a critical role for the RLV virus in the transformation. As was proposed in numerous previous published experiments using RLV infected rat cells, these cells provided a well tested sensitive and highly reproducible system for rapidly assaying in coded double blind tests the carcinogenic activity of chemicals

known to have carcinogenic activity. A number of suspect chemicals, particularly food color dyes, were found to have oncogenic activity in vivo after having been found positive in vitro. Freeman and his associates also described significant enhancement of transformation by chemicals and papovaviruses in otherwise stable rat cells following derepression of endogenous rat type C virus expressions using IdU prior to chemical treatment, thus showing that endogenous xenotropic type virus could also serve as a cocarcinogen.

Xenotropic viruses oncogenic in vitro? Price et al. recently observed malignant transformation in each of 4 lines of rat cells infected with C57L/J xenotropic virus: the transformation occurred at 6 and 7 subculture levels. Uninfected sister control lines did not transform. The significance and viral specificity of this observation must now be determined with the use of type specific neutralizing antisera.

In vivo cocarcinogenesis in hamsters. In previous annual reports VCB and STS scientists reported activation of p30 and RNA expressions of type C viruses in mouse and hamster tumors induced by carcinogencic chemicals (DMBA) and by DNA tumor viruses (Whitmire, Huebner, Lane and others.)

Recently cocarcinogenic interactions of a papovavirus and type C RNA virus were observed. Huebner, Lane, Hill (NCI), Gilden (Flow), and Takemoto (NIAID) demonstrated polyoma-like T antigens in visceral lymphomas which developed in hamsters under the following circumstances:

1. Following scarification of newborn hamsters with extracts of hamster trichoepitheliomas (Graffi warts.)

2. Spontaneous lymphomas occur in Graffi hamsters in association with several warty lesions in the colony (Gardner, USC.)

3. Intraperitoneal inoculation in NB and weanling hamsters of extracts from bovine papilloma induced cutaneous and subcutaneous tumors which contain large amounts of induced hamster type C virus (HaLV) (Huebner and Gilden.)

4. University of Tennessee lymphosarcoma epidemics; horizontal transmission across cages in a special room in a laboratory building (Ambrose and Coggins.) At least 4 epidemics of horizontally transmitted virus have been recorded at the University of Tennessee laboratory and in the animal laboratories of the Viral Carcinogenesis Branch. The behavior of the virus is similar to that of polyoma and the hamster tumors exhibit polyoma T antigen and tumored hamsters have polyoma T antibody, however, the easily transmitted virus lacks the virion (V) antigen of polyoma, thus this appears to be a distinct new virus of the type

observed in EM by Graffi in East Berlin. Although the virus can be neutralized by sera from hamsters carrying the lymphosarcoma, it is not neutralized by antibody to HaLV. These fascinating situations make it possible to study the interactions of HaLV endogenous type C RNA virus and the papovaviruses; the latter, like DMBA, clearly induces high level expressions of HaLV. Although this complex virus induced disease has occurred so far only in hamsters, the close serological relationship of the hamster T antigen to that of polyoma discloses a previously undetected antigenic sharing which suggests that the hamster virus and its T antigens must now be compared with the papoviruses of both man and monkeys which also show T antigen sharing.

Summaries of Major Findings In VCB

1. Dr. Wade Parks (Viral Genetics Section) and his group in studying type C virus polynucleotides found that immunologic typing using radioimmune competition assays provides evidence for relatedness and differences that cannot be measured by nucleic acid hybridization; thus p's 10, 12, 15 and 70 all have marked type specificity capable of distinguishing the heterogenous viruses in different mouse strains. The noninfectious rat type C viruses on the other hand appear to be remarkably homogenous.

Immunoassays of mammary tumor virus MTV revealed a group specific MMTV p14 which proved useful in showing a high correlation between MMTV polypeptide expressions and mammary carcinoma; early expression forecasting early mammary tumors. Adequate reagents for these studies were made possible by the find that glucocorticoid stimulation produced 10-fold increases in MMTV nucleic acid proteins and also extracellular virions in mammary tumor cell cultures.

2. Dr. Aaronson (Molecular Biology Section) and associates in extending their studies of cellular (genetic) regulation of endogenous ecotropic murine leukemia viruses to that of the endogenous xenotropic viruses found that the latter viruses are not regulated by the well known two genes which regulate the ecotropic viruses. Preliminary results of mendelian crosses between the high (NZB) and low (NIH Swiss) xenotropic expressions have revealed a partially dominant gene (in NZB) determining virus expression. Aaronson's group was the first to show that natural neutralizing antibodies to the xenotropic viruses was in some strains high (NZB) and in others quite low (NIH Swiss), thus studies for segregation of genetically controlled immune responses were also made possible.

Earlier in FY 1975, this group also tested the oncogenicity of "N" tropic viruses of C58 and BALB/c origins in appropriate N type newborn NIH Swiss mice; each produced a different type of leukemia in significant numbers thus establishing that both types of ecotropic MuLV's have oncogenic potential. Development of an assay for the type specific p12's (12000 MW) of type C RNA viruses made it possible to distinguish the B and N tropic

from the xenotropic viruses of BALB/c cells. Similarly the p12's of several strains of gibbon virus were found to be distinct from each other and distinct also from the otherwise similar woolly monkey virus. Of considerable importance, however, was the further finding that the newly isolated Gallo virus contained the same p12 as well as p30 of the woolly virus.

3. Dr. Berge Hampar (Solid Tumor Virus Section) reports that EBV virus was progressively activated in synchronized human lymphoblastoid cells during the early S phase by thymidine analogues; this was at a time (the S-1 period) which corresponded to the time of replication of the repressed viral genome. Protein inhibitors also activated the genome but only 6 or more hours after removal of the inhibitor.

Studies of synchronized and non-synchronized cells were critical to repeatable activation of the EBV genome. Studies of the role of herpes simplex in cell transformation led to scepticism that hamster cells which are susceptible to spontaneous transformation were in fact transformed by the HSV.

4. Dr. Gary Kelloff (in collaboration with scientists at Contract 33248). Cell mediated assays (foot pad reaction, lymphocyte blastogenesis and lymphocyte cytotoxicity) were developed and utilized to assess immune responses to type C MuLV vaccines in mice. The chief objectives are to assess the role of various cell mediated responses in prevention of natural type C RNA viral replication and spontaneous cancers.

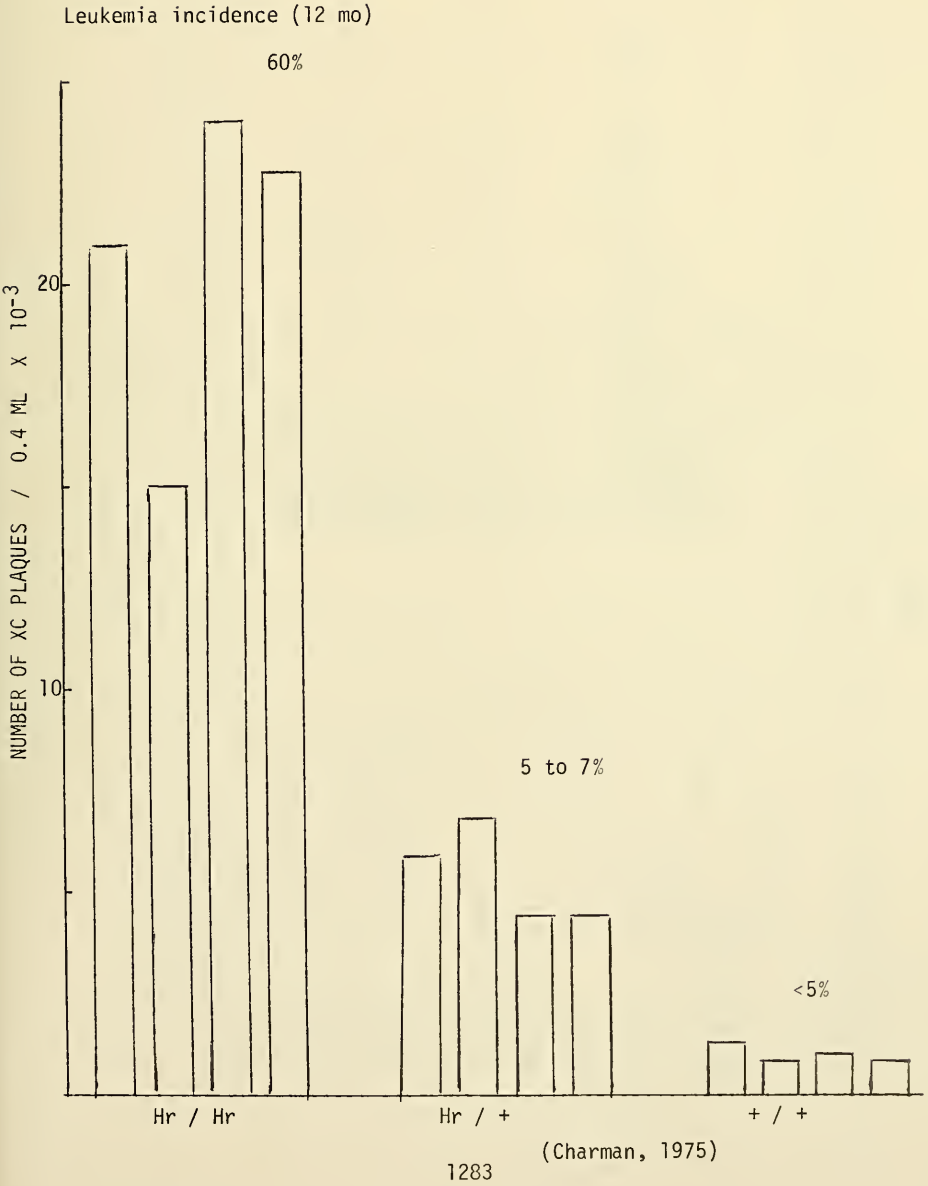
5. Drs. Hartley, Rowe and associates made major contributions in FY 1975. Dr. Rowe and associates proved that a single virus inducing locus in AKR mice, Akv-1, contains viral genetic material for producing the AKR virus; the DNA copy sequences correlated perfectly with the presence of Akv-1 in mice segregating for this locus, thus firmly establishing the virogene-oncogene theory for this strain of mouse, and probably for mice in general, since virtually all the mice infected with ecotropic viruses have the AKR type of natural virus.

Dr. Hartley and her associates observed a new type C RNA virus population called amphitropic being able to replicate in N type cells and in cells of a variety of other species, thus it has properties of both the ecotropic and xenotropic viruses.

6. Dr. Paul Arnstein, collaborating with Drs. Lennette, Riggs, Huebner and others, played a significant role in the isolation of a number of type C xenotropic viruses most notably from C57L/J, NIH Swiss, and nude mice. More recently he has studied the possible use of glutaraldehyde inactivated cells chronically infected with endogenous ecotropic type C viruses for vaccine prevention of cancer. Several types of immunizations reduced by 60-80% the efficiency of transplantation of a virus negative syngeneic fibrosarcoma. However, 2 doses of killed corynebacteria was equally as effective.

7. Dr. Padman Sarma (Ecology and Epizoology Section) recently isolated a type C virus from two outbreaks of a fatal reticuloendotheliosis (RE) in turkeys. The viruses in culture (turkey and chick cells) had the usual properties of type C viruses but were antigenically unrelated to avian leukosis viruses and were antigenically identical with REV strain T. In collaboration with Dr. P. Paul (University of Minnesota) Dr. Sarma has found the virus was oncogenic in young turkeys.

Fig 1. Tail Virus Titers at 6 months of age in Congenic HRS Mice



(Charman, 1975)

TABLE 1 (a)

Endogenous XENOTROPIC type C viruses (noninfectious for cells of natural host; infectious for cells of other species. Transmitted genetically?)

	Virus Strains	Investigators
Mouse[1]	NZB[2]	LEVY & PINCUS, 1970
	NIH Swiss[2] (AT124)	TODARO et al., 1973
	BALB/c-2	STEPHENSON et al., 1974
	C57L [2]	ARNSTEIN, in preparation
	C57BL/6	LEVY, 1973; BENVENISTE et al., 1974
Hamster	HALV-1	FREEMAN et al., 1974
Rat	RALV-1	KLEMENT et al., 1973
Cat	RD114	McALLISTER et al., 1972; FISCHINGER et al., 1973; LIVINGSTON & TODARO, 1973; SARMA et al., 1973
Chicken	Strain E	WEISS, 1973
Swine[2]	PK15	MOENNIG et al., 1974; TODARO et al., 1974
Baboon[2]	BabLV	KALTER et al., 1973; BENVENISTE et al., 1974 TODARO et al., 1974
Human[2]?	Baboon virus-related p30	SHERR & TODARO, 1974

[1]All laboratory mouse strains have a serologically very similar xenotropic virus; many have ecotropic viruses in addition, strain 129/J produced tropic neutralizing T antibody, p30 and p70 antigens but no infectious virus has been isolated.

[2]Animals in which xenotropic virus is only type of virus demonstrated or suspected.

TABLE 1 (b)

Endogenous ECOTROPIC viruses (infectious [tropic] for cells of natural host; some transmitted genetically, others epigenetically)

	Virus Strains	Investigators
Mouse	AKR virus	GROSS, 1961
	C57BL/6-RADLV	LIEBERMAN & KAPLAN, 1959
	BALB/c N-tropic	AARONSON et al., 1969; HARTLEY et al., 1970
	BALB/c B-tropic	HARTLEY et al., 1970
	MTV milk agent	BITTNER, 1936
Wild Mouse	LC & 1504E strains	GARDNER et al., 1971
Hamster	HALV-2 [MSV(HALV)]	KELLOFF et al., 1970
Rat	RALV-2 [MSV(0)]	TING, 1968; BERGS et al., 1972
Cat	FeLV 1, 2, 3	THEILEN et al., 1968; RICKARD et al., 1969;
	FSV	GARDNER et al., 1970; SARMA et al., 1970

TABLE 1 (c)

EXOGENOUS RNA Tumor Viruses (highly infectious for original hosts but often also for heterotypic hosts)

		Investigators
Vertically transmitted through maternal reproductive tract and experimental transmission		
ALV - avian leukosis viruses:	Strains A, B, C, D	RUBIN et al., 1961
MuLV - murine leukemia viruses:	GLV, FLV, MLV, RLV	GROSS, 1951 & 1958; FRIEND, 1957; MOLONEY, 1960; RAUSCHER, 1962
Horizontally transmitted through excretions and/or nursing of newborns		
Chicken - ALV Strains A and B		BURMESTER & WATERS, 1955: BEARD, 1957
Mouse mammary tumor virus (MTV)		BITTNER, 1936; ANDERVONT & DUNN; 1948; HESTON, 1965
Cat feline leukemia viruses (FeLV's)		HARDY et al., 1973; JARRETT et al., 1973
Gibbon Ape & Woolly Monkey viruses		KAWAKAMI et al., 1972; PARKS et al., 1973; GILDEN et al., 1974

TABLE 2

EFFECT OF HOMOZYGOUS REGULATING GENES IN C57Bl/10 AND LAKE CASITAS (LC) WILD MOUSE VIRUS EXPRESSIONS IN F_1 CROSSES AND BACKCROSSES TO C57Bl/10/Sn ASSAY USING SPLEEN p30 ANTIGEN

Strain	Number Positive (1:20 Final Dilution)	% Positive	
B10	1/53	1.9	
LC	146/180	81	
B10 X LC F_1	1/113	<1	1.4
LC X B10 F_1	1/32	3.1	
B10 X (B10 X LC) F_1	0/56	0	1.14
B10 X (LC X B10) F_1	1/32	3.1	
(B10 X LC) F_1 X B10	1/55	1.8	3.6
(LC X B10) F_1 X B10	3/56	5.3	
B10 X (B10 X LC) F_2	1/29	3.4	2.6
B10 X (LC X B10) F_2	0/9	0	
(LC X B10) F_2 X B10	0/22	0	0

Note: At a year of age the B10F_1's and backcrosses have virtually no lymphomas or paralytic disease.

M.B. Gardner, V. Klement, B.E. Henderson, H. Meier, J.D. Estes, R.J. Huebner (In Press)

TABLE 3

NEUTRALIZATION OF SPECIFIC VIRUSES***

Vaccine made from	AKR			SLV			WM			RLV		
	10	20	40*	10	20	40	10	20	40	10	20	40
AKR (virus)	100**	98	75	6	0	0	0	0	0	0	0	0
SLV	0	0	0	100	99	92		NT		0	0	0
Wild Mouse	0	0	0	0	0	0	100	99	70		NT	
RLV	29	0	0	0	0	0	0	0	0	73	41	0

* Reciprocal of serum dilution.
** Percent foci neutralized.
*** By pooled sera of mice given 3-4 inoculations as weanlings of formalin-inactivated banded virus vaccines.

TABLE 4

TYPE SPECIFICITY OF NEUTRALIZING ANTIBODIES PRODUCED IN SERA[1] OF MICE BY GROSS AND FMR PSEUDOTYPE SARCOMA VIRUSES

Virus[2]	Dilution	Serum[3]	Dilution	No. with Sarcomas[4]	
GL-MSV	1:10	GL-MSV	1:10	0/16	(40 days)
GL-MSV	1:10	RL-MSV		14/14	(15 days)
FL-MSV	1:50	GL-MSV		14/14	(10 days)
FL-MSV	1:50	FL-MSV		0/16	(40 days)
ML-MSV	1:100	GL-MSV		16/16	(10 days)
ML-MSV	1:100	ML-MSV		0/16	(40 days)
RL-MSV	1:100	GL-MSV		15/15	(12 days)
FL-MSV	1:100	RL-MSV	↓	0/16	(40 days)

[1] All antisera from NIH Swiss mice infected with pseudotype sarcoma virus.

[2] Virus - 10% clarified extract of sarcoma tissue, diluted to contain at least 40 sarcoma-inducing units.

[3] Taken 62 days after injection from mice in which sarcomas had regressed.

[4] Periods of observation.

TABLE 5
TITERS OF TYPE C VIRUS IN TAILS* OF VACCINATED** AND UNVACCINATED SWR x AKR F_1's

	Vaccinated	Virus Titers		Unvaccinated Controls	Virus Titers
Exp 79	TC 1[†]	$<10^1$	Exp 80	TC 3	$>10^4$
	5	10^1		6	$>10^4$
	6	$<10^1$		7	$>10^4$
	8	$<10^3$			
	9	10^3			
Exp 91	TC 1	10^4	Exp 92	TC 1	$>10^4$
	5	10^2		4	$>10^4$
	6	10^1		7	$>10^4$
	10	$<10^2$		9	$>10^4$
	12	$<10^1$		2	$>10^4$
Average		$<10^2$			$>10^4$

*Tails of F_1's taken at 40 to 58 days of age (10% extracts).

**Three injections, banded formalin-killed (GLV) virus vaccine given to SWR females at 14-day intervals. Vaccinated newborns challenged with MSV(GLV); no tumors produced.

[†] TC = Toe clip

NOTE: 5 of 10 vaccinated mice had one log or less virus.

Serial No. ZO1-CP-04924-02-VC
1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunoprevention of Spontaneous Tumors with Viral and Cellular Vaccines.

Previous Serial Number: Same

Principal Investigator: Dr. Gary J. Kelloff

Other Investigators: Dr. Robert J. Huebner
Mr. Worth I. Capps
Mr. Roy Trimmer
Ms. Pat Johnson
Mr. W. T. Lane

Cooperating Units: Outside NIH:

Dr. Robert Peters, Dr. Robert Donahoe, Dr. Bernard Sass, Dr. Robert Nims, Microbiological Associates, Walkersville, Maryland

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

(A) Development and utilization of assays for monitoring cell mediated and humoral immunity in the mouse to type C virus and type C viral antigens and non-viral antigens in cellular vaccines prepared from syngeneic tumor cell lines.

(B) Determination of the optimal production, fixation, handling and storage of inactivated type C virus vaccines for maintenance of immunogenicity. Determination of the immunogenicity and oncogenicity of naturally occurring, live type C virus used as a vaccine in adult laboratory animals.

(C) Determination of whether type C virus vaccines can prevent spontaneous tumors in mice known to have measurable humoral and cellular immune responsiveness to the viral immunogens.

(D) Determination of the immune competence of laboratory animals with aging and with development of naturally occurring neoplasms and the effect of transfer of syngeneic immunocompetent cells on the incidence of naturally occurring neoplasms.

Methods Employed and Major Findings:

A footpad assay for measuring delayed type hypersensitivity to type-C virus has been developed (3). This test has shown that the subcutaneous route of immunization for the induction of DTH is superior to the i.p. route and that adjuvant is required. The test has been shown to be specific enough to distinguish murine type C viruses differing in their envelope subtypes, shows a graded increasing response to homologous virus with immunizations of virus up to 300 μg/mouse and does not react with obvious control antigens (cell extracts, fetal calf serum, etc.). The definitive proof of the viral specific nature of these measured responses is the ability to elicit this DTH response by challenge of virus immunized animals with purified polypeptides of the virus, gp69/71. Having established the viral specific nature of this footpad response, the correlation of this response with immunity to virus challenge, tumor cell challenge, and naturally occurring neoplasms is currently under study. Clearly, this assay will allow us to continually monitor the immune status of animals under study in long term vaccine experiments. An in vitro assay to monitor cellular immunity to type-C virus vaccine was essential to allow the simultaneous study of the host's lymphocyte reactivity to a variety of antigens, and was desirable for serial monitoring of animals as an adjunct to the footpad assay. These investigations have shown for the first time that cultured immune lymphocytes give a blastogenic response when exposed to inactivated type C virus (4). The doses needed to immunize the animal (200μg) have been found to be considerably higher than those needed to elicit the footpad response. The viral specific nature of this measured immune response has also been documented by different viral subtypes and purified polypeptides of the virus. A cell mediated cytotoxicity assay has been developed from animals immunized with inactivated type C virus vaccines. These lymphocytes show specific lysis of syngeneic target cells infected with type C virus as compared to the same syngeneic target cells that are not infected. The amount of specific lysis has to date been considered quite low but statistically significant. Experiments are in progress in attempts to enhance this specific lysis, as are experiments to correlate this cytotoxic response with immunity to virus challenge and tumor cell challenge. Weanling B/c mice immunized with a total dose of 200μg of R-MLV and AKR-MLV vaccines have shown neutralizing activity (1:20) specific for homologous immunizing virus. Immunization with lesser doses or subcutaneous immunization was not effective in eliciting neutralizing antibodies. The development of these assays for cellular immunity to type C virus and the ability to measure neutralizing antibody to type C virus provide formidable tools to design and monitor experiments aimed at immunoprevention of spontaneous tumors.

All type C viral vaccines under study have been evaluated by the following parameters: protein mass, CF titer before and after tween ether treatment, infectivity by XC test, reverse transcriptase activity, SPAT assay (1) to determine residual in vivo infectivity, and their ability to induce neutralizing antibody. These studies have determined the optimal means of fixation, handling and storage of the vaccine to maintain maximal immunogenic activity and allow standardization of each new vaccine lot. Additionally, an evaluation of the immunogenicity and oncogenicity of live type C virus vaccines is in progress. Although laboratory strains of murine leukemia virus such as R-MLV have been shown to be oncogenic when inoculated into adult animals, there is no evidence yet that naturally occurring leukemia viruses are oncogenic when inoculated into adults. We have found that inoculation of 10^4 SPAT (newborn) doses of B/c leukemia virus into adult animals will not successfully establish a splenic infection and there is preliminary evidence that these animals respond immunogenically to this virus. Long term oncogenicity and immunogenicity studies in adult animals are planned to evaluate these live virus vaccines. It is also of interest that when newborn animals are inoculated with this live B/c leukemia virus and splenic infection is established, then immunosuppression can be shown to occur far in advance to leukemia development. This suggests that immunosuppression from natural B/c-LV expression might account for natural leukemogenesis. In following these leads, studies are planned to assess whether natural B/c-LV expression is accompanied by immunosuppression in the aging animal and whether early immunization of adults with type C virus can diminish or eradicate the natural splenic infection.

Experiments have been done and more extensive experiments are in progress to prove that immunization with the type C virus vaccines can protect the host against challenge with exogenous murine leukemia virus. These experiments include envelope type specific controls (i.e. R-MLV and G-MLV immunization and R-MLV and B/c-MLV challenge). Preliminary experiments suggest that cross-protection to different envelope subtypes can be induced with these vaccines. The availability of B/c-MLV stocks of titers of 10^{6}-7 are now available and challenge experiments in progress will conclusively answer the question of cross-protection. This protection will be correlated with the immunoassay results obtained on these same animals. These experiments will be followed by viral vaccine immunization and challenge with tumor cell lines and tumor transplant lines (low passage) with and without type C virus. Studies are in progress with immunized animals which will be held for development of spontaneous tumors. As vaccine becomes available young animals (3 month and 9 month old) will be immunized and monitored for immunity and spontaneous tumor development.

Tumor dose kinetics of two spontaneously transformed B/c cell lines have been determined. Preparation of cellular vaccines from these lines and immunization with these vaccines have conferred complete protection to challenge with the homologous live tumor cells. These studies are being followed up by experiments to determine the importance of type C virus in these cellular vaccines. Primary and secondary transplants of spontaneous tumors of various histologic types are being prepared for use as cellular

vaccines to determine the importance of their histologic specificity as well as their endogenous type C virus expression. Our experiments indicate that the footpad assay and the lymphocyte transformation assay will be adaptable for use with extracts of cellular vaccines, so that these tests can also be used to monitor animals under study with cellular vaccines.

Studies to assess the immune competence of laboratory mice with aging and with the development of naturally occurring neoplasms are planned, and the effect of transfer of syngeneic immunocompetent cells from young hosts on these two parameters will be studied, as well as the effect of the adaptive transfer of lymphocytes specifically immune to type C virus.

Significance to Biomedical Research and to the Program of the Institute:

The vaccine studies are designed to ultimately determine whether type C viral vaccines and cellular vaccines can be used in the immunoprevention of spontaneously occurring neoplasms.

Proposed Course:

Continuation of studies outlined above.

Honors and Awards:

None

Publications:

1. Peters, R.L., Spahn, G.J., Rabstein, L.S., Huebner, R.J., and Kelloff, G.J.: Sensitive in vivo assay for detection of murine leukemia viruses. Appl. Microbiol. 28: 614-617, 1974.

2. Peters, R.L., Rabstein, L.S., VanVleck, R., Kelloff, G.J., and Huebner, R.J.: Naturally occurring sarcoma virus of the BALB/cCr mouse. J. Nat. Cancer Inst. 53: 1725-1729, 1974.

3. Peters, R.L., Donahoe, R.M., and Kelloff, G.J.: An assay in the mouse for delayed type hypersensitivity to murine leukemia virus. J. Nat. Cancer Inst. (In Press)

4. Donahoe, R.M., Peters, R.L., VanVleck, R., Huebner, R.J., and Kelloff, G.J.: Development of lymphocyte transformation microassay in the mouse to murine leukemia virus. J. Nat. Cancer Inst. (In Press)

5. Sass, B., Rabstein, L.S., Madison, R., Nims, R.M., Peters, R.L., and Kelloff, G.J.: Incidence of spontaneous neoplasms in 344 SPF Fischer rats throughout the natural lifespan. J. Nat. Cancer Inst. (In Press)

Serial No. ZO1-CP-04925-04-VC

1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Ecology and Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of type C RNA tumor viruses.

Previous Serial Numver: Same

Principal Investigator: Dr. Padman S. Sarma

Other Investigators: Dr. Robert J. Huebner, VCB, VO, DCCP, NCI
Mr. Paul Hill, VCB, VO, DCCP, NCI

Cooperating Units: Inside NIH:

Dr. Adi Gazdar, VLLB, VO, DCCP, NCI

Outside NIH:

Dr. L.B. Crittenden, USDA, Beltsville, Maryland
Dr. M. Gardner, USC School of Medicine, Los Angeles, California
Dr. R. Gilden, Flow Laboratories, Rockville, Maryland
Dr. R. Mellors, Hospital for Special Surgery, New York, New York
Dr. N.K. Mishra, Microbiological Associates, Bethesda, Maryland
Dr. P. Paul, University of Minnesota, St. Paul, Minnesota
Dr. P. Reddy, Microbiological Associates, Bethesda, Maryland
Dr. M. Van Der Maaten, National Animal Disease Center, Ames, Iowa
Dr. G. Theilen, University of California, Davis, California

Man Years:

Total:	7.0
Professional:	2.0
Other:	5.0

Objectives:

It is becoming increasingly apparent that many vertebrates including man carry information in their genomes for complete RNA tumor virus expression including oncogenic potential. This project is concerned with basic studies on the demonstration, isolation and characterization of such covert as well as overt type C RNA viruses from diverse avian and mammalian species, including man. A large portion of our present efforts is directed towards the development and use of sensitive in vitro systems for studying the prevalence and natural behavior of such viruses. Diverse approaches are being intensively applied to animal and human tumor systems in efforts to 'turn on' or rescue overt expressions of viral genomes.

Major Findings:

Differential host range of viruses of feline leukemia sarcoma complex: Selected strains of feline leukemia and sarcoma viruses of subgroups A, B and C show a differential pattern in their ability to cross species barrier and productively infect cells of heterologous host species. A virus of subgroup B showed the widest host range; it caused productive infection of cells of diverse host species including cells from cat, human, monkey, dog, bovine, pig, and hamster species. Two virus strains of subgroup A showed the narrowest host range; of the cells of several mammalian species examined, they only caused productive infection of cat and dog cells. Preliminary studies indicated that certain other strains of subgroup A viruses cause productive infection of whole human embryo cells. One strain of subgroup C virus examined showed a host range that was intermediate between that of A and B subgroup viruses. This strain caused productive infection of cat, dog, and certain human cells. In addition, the subgroup C virus caused productive infection of guinea pig cells found to be resistant to subgroup A as well as subgroup B virus strains examined.

Preliminary studies suggested that certain, but not all, virus mixtures of A and B viruses can be purified into B type by passage of virus in heterologous human cells. Present studies are designed to elucidate the factor(s) that may govern the differential susceptibilities of heterologous host cells to the described strains of feline viruses.

Isolation and characterization of viruses from natural outbreaks of reticuloendotheliosis in turkeys: The natural occurrence of turkey leukosis and the prevalence of type C viruses in turkeys are unknown. Although the reticuloendotheliosis virus (REV), strain T, was believed to have been isolated from an adult turkey with leukosis-like lesions, its origin and role in turkey leukosis have been in considerable doubt since the virus was derived after in vivo passages of affected turkey tissues in chickens.

We recently succeeded in isolating type C RNA viruses in vitro from two outbreaks of a fatal neoplastic disease in turkeys, diagnosed by us as reticuloendotheliosis. The virus has a density of 1.16 g/cc in sucrose

gradients, has a DNA polymerase capable of using both endogenous and exogenous (synthetic) templates, and is infectious in vitro for turkey and chick cells. The culture-propagated virus is oncogenic for turkeys. The virus isolates are unrelated to avian leukosis virus and are antigenically identical with REV Strain T. Our studies thus suggest REV may be a causative agent of naturally-occurring fatal leukosis in turkeys.

Equine lymphosarcoma - Test of cultured cells for type C virus: We have established a fibroblastic cell line from the lymphosarcoma tissues of a horse (collaboration with Dr. G. Theilen). The cell line does not contain type C virus as determined by electron microscopy (Dr. Vernon) and DNA polymerase assays. Attempts were made to isolate virus by passage of culture fluids into normal cultures of homologous and heterologous species, before and after IdU treatment. The IdU-treated cultures were also co-cultivated with such normal cells. These studies did not provide evidence to suggest the presence of a type C virus in any of the cultures. Cell pack antigens of the cells derived from equine tumor did not contain demonstrable gs-3 antigen as determined by CF tests.

Attempts to induce an endogenous type C virus of certain avian species: Attempts were made to detect and isolate a type C virus in apparently normal embryo cultures of turkey and partridge cells. Virus induction was attempted with IdU and dexamethazone followed by cocultivations with a variety of avian cells (turkey, chicken, partridge, quail) and hamster tumor cells containing defective RSV genome. These preliminary experiments did not suggest the induction or release of a type C RNA virus from turkey and partridge cells as determined by DNA polymerase assays, electron microscopy, and virus isolation methods.

Virological studies of Lupus Erythymatosus: In a collaborative study with Dr. Robert Mellors, we succeeded in establishing a cell line from spleen biopsy tissue derived from a woman with systemic Lupus Erythymatosus. Our preliminary virus screening and virus induction-cell cocultivation techniques have failed to reveal the presence of demonstrable type C virus.

Attempts to isolate type C virus from porcine tumors: Four separate cell strains were established from two cases of pig malignant nephromas and two of pig lymphosarcomas. One line derived from nephrosarcoma was positive for gs-3 antigen by CF test (titer 1:4). However, the cells were negative for type C virus by electron microscopy and DNA polymerase assays. We failed to rescue a type C virus from two of the cell lines by treatment of these cells with IdU followed by cocultivation of these cells with cells of human, monkey, dog, guinea pig, dolphin, rat, hamster, bovine, and caprine species. We also failed to isolate a virus by inoculation of secondary pig embryonic kidney cells with Moloney concentrates prepared from freshly excised tumors. These studies suggest that although the pig type C virus can be demonstrated in certain continuous pig cell lines, such as PK 15 (American Type Culture Collection), naturally occurring pig tumors do not reveal overt expression of pig type C virus.

Studies on bovine leukemia: Studies of several investigators have suggested the occurrence of type C-like virus particles in cattle with lymphosarcoma. This virus designated "bovine type C virus" has not been isolated and characterized for its biologic, antigenic and biochemical properties. In collaboration with Dr. S. Dutta of the University of Maryland, we undertook intensive studies to determine if apparently normal cells we derived from leukemic cattle can be induced to release a type C virus by treatment with halogenated pyrimidine, 5-iododeoxyuridine (IdU). Two fibroblastic cell lines derived from buffy coats of leukemic cattle and one normal bovine embryonic kidney cell line were treated with IdU for 48 hours followed by cell cocultivations with the following cultures: RD, whole human embryo, bovine kidney, dog embryo, and sheep kidney. Although occasional bursts of RNA-dependent DNA polymerase activities were noted, type C virus was not demonstrable in these cultures by electron microscopy and virus isolation techniques.

In more recent studies, in collaboration with Dr. Van Der Maaten, we are characterizing a virus which he has described as a bovine type C virus derived from buffy coat cultures of cattle with lymphosarcoma (J. Natl. Cancer Inst. 52:491-497, 1974).

A virus is being continuously produced by two of five cell lines he provided. Virus derived from one of the virus positive lines was characterized. The virus has a density of 1.14 g/cc in sucrose and exhibits an RNA-dependent DNA polymerase (RDP) activity. The enzyme prefers manganese over magnesium both with exogenous template (Oligo dT.poly rA) and endogenous template. The peak enzyme activity is produced at 0.01 mM Mn++ ion concentration whereas the optimal activity is obtained at 1 mM concentration of Mg++. The endogenous reaction is RNase sensitive and resistant to actinomycin D. The end product of the short endogenous reaction migrates with 70S RNA indicating that the DNA product is covalently linked with 70S RNA. The template-primer preference was found to be in the following order: $dT_{12-18}.rA$, $dG_{12-18}.rC$ and poly dA.dT. Taken together, this indicates that RDP of this virus behaves like a typical type C virus enzyme as opposed to type B viral enzyme or cellular "weissback type" polymerase.

A radiolabelled, single-stranded DNA transcript of the 70S RNA of this virus was prepared. Molecular hybridization experiments were performed using normal lamb spleen DNA, normal bovine cell DNA, human placental DNA, and bovine tumor cell DNA. The DNA transcript of virus hybridized extensively (80%) with normal lamb spleen DNA but showed only limited hybridization (20%) with normal bovine cell DNA and bovine tumor cell DNA. The hybridization with bovine cell DNA appears to be non-specific since 20% of the DNA transcript also hybridized with poly A. In order to avoid non-specific hybridization, we have prepared viral RNA reverse transcript from which all poly A-binding sequences have been removed. We are now carrying out the hybridization experiments using this probe. This reverse transcript may be much more representative of the 70S viral RNA and may thus provide more conclusive data regarding the origin of this virus.

The virus-producing cell line was found to be lamb cells by immunofluorescent technique and by chromosome analysis (Dr. C.S. Stulberg). The culture we received from Dr. Van Der Maaten was originally started as a mixture of bovine buffy coat cells and embryonic lamb spleen cells. Quantitative studies with the virus have been difficult since virus production from the lamb cells is low. Various mammalian cell cultures we have tested appear to be resistant to productive infection with the virus. The cultures tested include Swiss mouse, LLCMK2, bat lung, BSC-1, duck embryo, buffalo lung, raccoon uterus, RD, rabbit epidermis, dog osteosarcoma, ferret embryo, whole human embryo, Osborne-Mendel strain of rat embryo, embryonic bovine kidney, goat oesophagus, Fischer rat placenta, and chimpanzee placenta.

Concentrated virus disrupted with ether as well as cell pack antigens of virus-producing fetal lamb spleen cultures do not contain detectable mammalian (gs-3) interspecies antigen as determined by CF test.

Further studies are planned to confirm and extend this finding (collaboration with Dr. R. Gilden).

Significance to Biomedical Reserach and the Program of the Institute:

Our studies performed during the reporting period provided important and much needed information on the prevalence and behavior of covert as well as overt type C RNA tumor viruses of cat, chicken, turkey, and man. We undertook studies to determine the natural occurrence and behavior of type C RNA viruses of cattle, pig, and certain other mammalian and avian species. These studies have provided valuable information on the natural occurrence, expression, and spread of the RNA tumor viral genomes and provide tools for further studies of oncogenic type C RNA virus in homologous and heterologous species including man.

Viruses of feline leukemia-sarcoma group revealed differences in their ability to cross species barrier and infect heterologous host cells. Subgroup B virus was found to be the most pathogenic in this respect. Possible implication of this finding in the natural horizontal spread of virus to other mammalian species remains to be determined. Factor(s) that govern the differential host range are presently unknown and are under study.

The exact origin of the only isolate of reticuloendotheliosis virus, strain T, has been in considerable doubt since its original discovery in 1958. Our isolations of a similar virus from naturally occurring cancers of the turkeys establish the important role of this pathogenic agent in naturally occurring cancers of turkeys.

The origin and natural host of the "bovine" type C virus is obscure. Our present preliminary studies suggest that genetic information of this virus may be present in lamb cells rather than in bovine cells.

Proposed Course:

We will continue our studies on the demonstration, isolation and characterization of endogenous and epigenetic type C RNA viruses of selected mammalian species (cat, man, sheep, and cattle). Sensitive in vitro methods will be developed for the detection, assay and characterization of newly described type C RNA viruses derived from cattle and turkeys.

Honors and Awards:

None

Publications:

Sarma, P.S. and Gazdar, A.F.: Recent progress in studies of mouse type C viruses. Current Topics in Microbiol. and Immunol. 68: 1-28, 1974.

Sarma, P.S., Log, T., Jain, D., Hill, P.R., Huebner, R.J.: Differential host range of viruses of feline leukemia-sarcoma complex. Virology 64: (in press).

Sarma, P.S., Jain, D., Hill, P.R.: In vitro host range of feline leukemia virus. In: Comparative Leukemia Research 1973, pp. 437-440 (in press).

Sarma, P.S., Jain, D.K., Mishra, N.K., Vernon, M.L., Paul, P.S., Pomeroy, B.S.: Isolation and characterization of viruses from natural outbreaks of reticuloendotheliosis in turkeys. J. Natl. Cancer Inst. (in press).

Serial No. Z01-CP-04926-01-VC
1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Poly-L-lysine inhibition of mouse spleen polyribosome protein synthesis *in vitro*.

Previous Serial Number: Same

Principal Investigator: Dr. F. H. Portugal

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, VO, DCCP, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

Complexes of proteins with nucleic acids are known to occur within cells and may be important for the control of translation, transcription or both. Studies have frequently been carried out with DNA and basic proteins or polypeptides based on the observation that basic proteins such as histones may be important in regulating the biological activity of DNA.

However, it is now established that polypeptides, specifically poly-L-lysine, also interact and bind to RNA as well as DNA. Basic poly-amino-acids may enhance the infectivity of various viral nucleic acids such as poliovirus RNA or may inhibit the replication of other viruses by binding to the RNA template thereby inhibiting replicase enzyme activity as in the case of foot-and-mouth disease virus.

Furthermore, it is known that both RNA and DNA containing viruses have long segments of approximately 200 nucleotides consisting exclusively of adenine. Since polymers of lysine are synthesized *in vitro* from oligonucleotide templates consisting of adenine it is possible that poly-L-lysine may have considerable importance in regulating viral activity if these viruses utilize this region of the genome.

Since the biochemical process of translation utilizes polysomes which contain RNA templates, I investigated the effects of poly-L-lysine on translation in vitro and the mechanism for this activity.

Methods Employed:

Free mouse spleen polyribosomes were isolated in the presence of rat liver supernatant to inhibit ribonuclease digestion of the nucleic acids. The pH 5 enzyme fraction was prepared from Sprague-Dawley rat liver. The complete system was dependent on the addition of polyribosomes,pH 5 enzyme fraction, an energy source, and GTP. The system was efficient and incorporated up to 160 pmoles/ml of leucine for 0.65 mg spleen ploy ribosome RNA.

Major Findings:

The addition of as little as 4 ug of poly-L-lysine (34,800) per ml of reaction produced 50% inhibition. More than 90% inhibition was attained at 10 ug. Puromycin at these concentrations gave essentially the same levels of inhibition. Poly-L-ornithine (105,000) gave similar results as did poly-L-arginine. The inhibition by poly-L-lysine was observed with chain lengths up to 100 residues. Chains of pentameric lysine (or smaller) had no activity. The same results were obtained with mouse liver polysomes as with the spleen.

The mechanism of activity was demonstrated in several ways. First, under conditions where in vitro protein synthesis was inhibited by 93%, the addition of increasing amounts of fresh polysomes overcame the inhibition. The efficiency was dependent on the levels of poly-L-lysine employed. Sucrose density analysis showed that during incubation the presence of poly-L-lysine resulted in less breakdown of the polysomes to monosome units.

In similar experiments, the addition of increasing amounts of the pH 5 enzyme fraction failed to overcome the inhibition produced by poly-L-lysine. Studies of amino acid acylation indicated that where levels of poly-L-lysine produced only 15% inhibition, more than 95% inhibition of leucine incorporation into protein occurred.

Finally, the possibility that poly-L-lysine inhibited in vitro protein synthesis by altering the cation requirements was tested by using polyanion analogs known to produce such effects in systems of in vitro synthesis which utilize MS 2 phage RNA templates. Neither spermine nor putrescine had any effects on the mouse spleen polysome system at comparable concentrations to those used for poly-L-lysine.

All of these studies are consistent with and support the idea that poly-L-lysine is a potent inhibitor of in vitro protein synthesis by combining with the RNA of the polysome and thereby blocking the translation process.

Significance to Biomedical Research and the Program of the Institute:

Oncogenic type C virus contains an RNA genome. Furthermore, there are suggestions that the genome contains polyadenine rich sequences. In addition, conditions which inhibit cellular protein synthesis (translation) result in a marked increase in type C virus production. Thus it is of interest to determine specifically what effects poly-L-lysine has on the translation of type C viral information and whether these effects are selective. Furthermore, it would be of interest to determine whether poly-L-lysine can block any steps leading to cell transformation. It would also be of interest to test the effects of poly-L-lysine on virus-induced tumors *in vivo*.

Proposed Course:

A. Utilizing radioimmune assays, the products of *in vitro* synthesis from mouse spleen polysomes in the presence of poly-L-lysine will be compared to the products produced in the absence of the inhibitor to determine whether poly-L-lysine selectively alters the production of type C viral proteins.

B. The studies of inhibition of *in vitro* protein synthesis by poly-L-lysine will be carried out using type C viral RNA templates to determine whether poly-L-lysine has the same activity with these templates as with cellular ones.

C. Attempts will be made to determine whether poly-L-lysine is a natural component of cells and to determine whether the levels of poly-L-lysine fluctuate under different biological conditions as do other polyamines.

Honors and Awards:

None.

Publications:

None.

Serial No. ZO1-CP-04928-03-VC
1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Viral Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunologic Studies of Virion-Associated Antigens in Natural Tissues

Previous Serial Number: Same

Principal Investigator: Dr. Wade P. Parks

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. Robert J. Goldberg, OADVO, DCCP, NCI
Dr. W. Heston, LBV, DCB, NCI
Dr. Robert J. Huebner, VCB, VO, DCCP, NCI
Dr. E.M. Scolnick, VLLB, VO, DCCP, NCI

Outside NIH:

Dr. Raymond Gilden, Flow Laboratories, Inc. Rockville, Maryland
Dr. Ronald Gillette, Meloy Laboratories, Inc., Springfield, Virginia
Dr. Hans Meier, Jackson Laboratory, Bar Harbor, Maine

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

(1) Detection and identification of reverse transcriptase-containing viruses in natural tissues employing biochemical, biologic and immunologic procedures. (2) To characterize virion surface proteins involved in naturally-occurring immune responses and to correlate cell surface antigens with virus expression. (3) Using expression of viral protein as indicators, to study the genetic basis for cellular mutation and transformation.

Methods Employed:

As described previously.

Major Findings:

1. Murine type C viruses constitute a heterogenous class of viruses as demonstrated by the immunologic reactions of glycoprotein (gp70) and low MW (p15, p12/10) polypeptides. Although the p12 and p10 are immunologically cross-reactive in Kirsten and Moloney strains and are the most type-specific type C polypeptides examined, the p15 and gp70 also have marked type-specific reactivity. The Friend and Rauscher strains were demonstrated to be only distantly related to Moloney virus by competition immunoassays with these proteins and xenotropic viruses were shown to be a novel group distinct from either the FMR or AKR-Gross types of MuLV. The ability to type MuLV strains is essential to delineate the roles of these viruses in vivo. Similarly, we are attempting to measure host immunologic responses to virion expression.

2. In contrast to the heterogeneity of the murine type C viruses, rat type C viruses constitute a single type of non-infectious virus. Eight different isolates were examined in p12/10 specific competition radioimmunoassay and found to be highly related. In those instances where nucleic acid hybridization was performed a similar high level of relatedness was noted, except when high levels of a novel set of nucleic acid sequences was packaged within the virion. These results suggest that immunologic typing may provide evidence of relatedness that cannot be measured by nucleic acid hybridization, at least in some mammalian cells.

3. Regulation of type B mouse mammary tumor virus sequences in murine cells was shown to involve a glucocorticoid-receptor interaction. The effect of the hormone-receptor complex was at the level of DNA RNA transcription and resulted in a highly specific increase in type B viral nucleic acid sequences but not of type C sequences. This 10-fold increase in nucleic acid following hormone treatment of mammary tumor cells in culture is reflected in comparable increases in MMTV-specified proteins and finally in extracellular virions. This technique of stimulating MMTV in tissue culture has been used as a source of virus and to investigate the mechanism of action of glucocorticoids. Characterization of the glucocorticoid receptor responsible for MMTV induction suggests it is similar in its structure and binding affinity to glucocorticoid-receptors in other mammalian cells.

4. Characterization of type B virion polypeptides and development of specific immunoassays has been extended from the gp52 and p27 to the low MW MMTV polypeptides. The MMTV p14 is group-specific in its reactivity measuring type B virus in mice from diverse geographic and different natural history settings. Immunoassays using this protein have greatly facilitated the analysis of milks and tissues for type B polypeptides and revealed a

very high correlation between MMTV viral polypeptide expression and mammary adenocarcinoma. In genetic crosses, early high expression of viral antigens correlates closely with propensity for developing mammary tumors in the first 12 months.

Significance to Biomedical Research and the Program of the Institute:

The regulation of expression of inherited viral genomes and/or chromosomally integrated epigenetic infections underpins both etiologic and pathogenesis studies of cancer biology. Towards this end, the use of viruses to study mammalian cell regulatory phenomena and factors which affect regulation such as hormones or carcinogens is of central importance. Since both type B and type C viruses are clearly involved in many murine cancers, both viruses must be investigated. Finally, the almost weekly novel findings in the murine, rat, cat and primate virus systems continually open new approaches to attempts to detect human viral information.

As a major part of the above, immunologically oriented studies of natural immune responses provide the most logical means of control of viral-associated disease. To measure humoral and cell-mediated responses specifically associated with virion polypeptides will greatly hasten immune control efforts such as vaccination.

Proposed Course:

Using recently characterized viral polypeptides, we propose to correlate specific virus expression with specific diseases in mice, rats, cats and gibbons both in vitro and in vivo systems. Major emphasis will be directed at type B viruses in mice and natural disease in cats and gibbons.

Efforts to measure host immune response to viral antigens and mechanisms of host resistance will be continued and expanded. This effort will characterize positive human responses to MMTV and subhuman responses to presumed epigenetic type C infections.

Honors and Awards:

None

Publications:

Parks, W.P., Howk, R.S., Scolnick, E.M., Oroszlan, S. and Gilden, R.V.: Immunochemical characterization of two major polypeptides from murine mammary tumor virus. J. Virol. 13: 1200-1210, 1974.

Scolnick, E.M., Parks, W.P.: Harvey sarcoma virus: A second murine type C sarcoma virus with rat genetic information. J. Virol. 13: 1211-1219, 1974.

Parks, W.P. and Scolnick, E.M.: Immunological cross reactions between two low molecular weight polypeptides from a murine type C virus. J. Virol. 14: 430-433, 1974.

Howk, R.S., Williams, D.R., Haberman, A.B., Parks, W.P. and Scolnick, E.M.: A comparison of the transcription of mouse cell chromatin by homologous RNA polymerase I and II. Cell 3: 15-22, 1974.

Parks, W.P., Scolnick, E.M. and Ransom, J.C.: Glucocorticoid induction of murine mammary tumor virus in vitro. Cold Spring Harbor Symposium 39: 1974, in press.

Scolnick, E.M., Goldberg, R.J. and Parks, W.P.: A biochemical and genetic analysis of mammalian RNA-containing sarcoma viruses. Cold Spring Harbor Symposium 39: 1974, in press.

Goldberg, R.J., Scolnick, E.M. and Parks, W.P.: Isolation of a primate type C virus from a lymphomatous baboon. Int. J. Cancer 14: 722-730, 1974.

Scolnick, E.M., Maryak, J.M. and Parks, W.P.: Levels of rat cellular RNA homologous to either Kirsten sarcoma virus or rat type C virus in cell lines derived from Osborne-Mendel rats. J. Virol. 14: 1435-1444, 1974.

Parks, W.P., Ransom, J.C., Young, H.A. and Scolnick, E.M.: Mammary tumor virus induction of glucocorticoid: characterization of specific transcriptional regulation. J. Biol. Chem., 1975, in press.

Young, H.A., Scolnick, E.M., and Parks, W.P.: Glucocorticoid-receptor interaction and induction of murine mammary tumor virus. J. Biol. Chem., 1975, in press.

Howk, R.S., Anisowicz, A., Silverman, A.Y., Parks, W.P. and Scolnick, E.M.: Distribution of murine type B and type C viral nucleic acid sequences in template active and template inactive chromatin. Cell, 1975, in press.

Parks, W.P. and Rapp, F.: Prospects for herpesvirus vaccination safety and efficacy considerations. Progress in Medical Virology, in press.

Parks, W.P., Noon, M.C., Gilden, R. and Scolnick, E.M.: Serologic studies with low molecular weight polypeptides from the Moloney strain of murine leukemia virus. J. Virol., 1975, in press.

Serial No. Z01-CP-04929-07-VC

1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Solid Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Herpesviruses: Immunologic and Biologic Studies in Relation to Human Tumors

Previous Serial Number: Same

Principal Investigator: Dr. Berge Hampar

Other Investigators: None

Cooperating Units: Inside NIH

Dr. George Vande Woude, VB, VO, DCCP, NCI
Dr. Ashley Brown, VCB, VO, DCCP, NCI

Outside NIH

Dr. J.G. Derge, Flow Laboratories, Inc., Rockville, Maryland
Dr. M. Nonoyama, Presbyterian-St. Luke's Hospital, Chicago, Illinois
Dr. G. Yerganian, Children's Cancer Research Foundation, Boston, Massachusetts
Dr. R. Okabe, Flow Laboratories, Inc., Rockville, Maryland

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(1) To study the mechanism of herpesvirus latency in human cells with emphasis on the Epstein Barr virus.

(2) To determine whether herpes simplex virus can transform cells in vitro.

Methods Employed:

Standard tissue culture, virologic, and biochemical methods were employed.

Major Findings:

(1) The state of the repressed EB virus genome in human lymphoblastoid cells: Previous studies employing synchronized human lymphoblastoid cells indicated that initiation of the virus activation sequence by thymidine analogues occurred during the (S-1 period) at a time which corresponded to the time of replication of the repressed virus genome. Additional studies described the programing of virus antigen synthesis following activation by 5-iododeoxyuridine (IdU). These studies have been extended using drugs other than thymidine analogues for initiating activation of the repressed EB virus genome. When synchronized nonproducer Raji cells or producer P3HR-1 cells were treated with protein inhibitors (cycloheximide or puromycin) or were made deficient in arginine, virus activation was initiated when the drugs were added during the S-1 period and removed after 5 hours. The time of addition of the drugs for initiation of the virus activation sequence was well delineated in synchronized cells and a significant difference in the percentage of activated cells was noted when the drugs were added at 1 hour into the S phase compared to their addition at 30 or 90 minutes into the S phase.

The time of removal of the protein inhibitor for virus antigen expression was also critical, and maximum expression of virus antigen(s) was noted when the inhibitors were removed at 6 hours into the S phase compared to removal at 5.5 hours or 6.5 hours. The critical time for removal of the protein inhibitor for activation was related to DNA synthesis in that the addition of ara-c or hydroxyurea at any time from 1 to 6 hours into the S phase prevented activation by cycloheximide or arginine deficiency. This finding was unexpected based on previous results using IdU where it was shown that inhibition of DNA synthesis at the time of removal of the IdU (2 hours into the S phase) did not prevent virus activation and subsequent appearance of virus antigens.

To summarize briefly, we found that activation of EB virus by inhibitors of protein synthesis required (a) addition of the inhibitor at 1 hour into the S phase which corresponds to the time of replication of the repressed virus genome and initiation of virus activation by IdU, and (b) removal of the inhibitor at 6 hours into the S phase which corresponded to completion of synthesis of an unidentified DNA sequence.

(2) EB virus activation induced by chemicals in synchronized vs. non-synchronized cells: Most attempts to activate EB virus by IdU have employed non-synchronized cells, and large variations have been noted between different cell lines in the frequency of activation. These variations have been attributed to various factors including the state of the repressed virus genome in different cells. The use of synchronized cells for virus activation suggests that the inability of certain drugs to activate virus when added to

non-synchronized cells may be related to the uptake or utilization of the drug rather than to the inherent resistance of the cells for activation by the drug in question. Synchronization of cells which are readily activated by IdU when added to non-synchronized cells indicates that the synchronization procedure, in itself, does not enhance the level of virus activation by the drug during any one cell cycle. Consequently, the role of synchronization is merely to delineate the stage during the cell cycle when specific events occur during and subsequent to activation of the repressed EB virus genome. By using synchronized cells, we were able to induce virus activation by drugs (e.g. arginine deficiency, cycloheximide) which could not induce activation in non-synchronized cells. This is consistent with the concept that virus activation by drugs added to non-synchronized cell populations requires that the cells be able to traverse the cell cycle to reach the critical point where virus activation is initiated. Drugs which inhibit the cells from traversing the cell cycle obviously will not activate virus in non-synchronized populations.

(3) Association of EB virus induced early antigen (EA) with chromosomes: Human lymphoblastoid cells carrying a repressed EB virus genome show a nuclear antigen (EBNA) detectable by complement-fixing immunofluorescence. The EBNA antigen is associated with chromatin material and is found on chromosomes of mitotic cells. The significance of this antigen is still unknown, although it does have similarities to the T antigens of DNA tumor viruses. The fact that EBNA is associated with chromatin has been cited as evidence that this antigen complex is somehow related to the mechanism of latency of the virus. Studies carried out in our laboratory indicate that the early antigen (EA) complex, which is the first antigen detectable in the virus productive cycle in activated cells is also associated with chromosomes in mitotic cells. Further, the synthesis of EA does not preclude the cells from reaching mitosis, although the possibility that the cells are able to successfully complete division has yet to be determined. Regardless, the fact that both EBNA and EA are associated with chromatin raises questions as to its significance with respect to the role of EBNA in maintaining the virus genome in a repressed state.

(4) Role of herpes simplex virus in cell transformation: An extensive literature has appeared indicating that transformed cells may appear in cell populations infected with UV irradiated or temperature-sensitive mutants of HSV, and attempts have been made to ascribe a direct role for the HSV genome in the transformation process. Unfortunately, convincing evidence that the herpesvirus genome is directly involved in transformation has not been presented. Studies have been undertaken in our laboratory to determine what role, if any, HSV plays in transformation of cells in vitro. During the past year we have established systems where transformants have been obtained following infection with inactivated HSV. At present, we have not been able to pinpoint the exact role of HSV in these transformations and detailed results are not yet available. Preliminary studies, however, suggest that HSV may not play a direct role in transformation of mouse cells in vitro.

Significance to Biomedical Research and the Program of the Institute:

Numerous studies suggest that herpesviruses may play a primary role in cell transformation and oncogenesis. Despite their number, none of these studies have definitively established a direct oncogenic role for the herpesvirus genome. The best available evidence is with Epstein-Barr virus which has been shown capable of transforming lymphoid cells in vitro resulting in long-term cell lines. Whether this type of transformation leads to oncogenesis is still undetermined. In the case of HSV, evidence for a direct oncogenic role is lacking although many investigators consider the appearance of transformed cells in cultures infected with inactivated HSV as supportive of an oncogenic role. It is imperative that efforts be made to either prove or exclude a role for herpesviruses in oncogenesis.

Proposed Course:

Studies will continue to determine the role of herpesviruses in oncogenesis. In the case of Epstein-Barr virus, the studies will be directed towards further delineating the state of the repressed virus genome in lymphoid cells and the mechanism of virus latency. Once this has been established, the studies will be directed towards the mechanism of the initial transformation step by the virus in lymphoid cells. Studies with HSV will continue employing cell systems developed during the past year. These studies are long-term in nature, as the appearance of transformed cells may take several months after infection with inactivated virus.

Honors and Awards:

None

Publications:

Nonoyama, M., Tanaka, A., Hampar, B., and Derge, J.G.: Latent genomes of Epstein-Barr virus. In Robinson, W.S. & Fox, C.F. (Eds.): Mechanisms of Virus Disease, ICN-UCLA Symposium on Molecular and Cellular Biology. New York, W.A. Benjamin, Inc. 1974, pp. 505-514.

Hampar, B., Derge, J.G., Tanaka, A., and Nonoyama, M.K.: Sequence of Epstein-Barr virus productive cycle in human lymphoblastoid cells. Cold Spring Harbor Symposium on Tumor Viruses. 1974 (In Press).

Hampar, B., Derge, J.G., Nonoyama, M., Chang, S-Y, Tagamets, M.A., and Showalter, S.D.: Programming of events in Epstein-Barr virus activated cells induced by 5-iododeoxyuridine. Virology 62: 71-89, 1974.

Serial No. Z01-CP-04930-04-VC
1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Field Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biology of Tumor Viruses in Naturally Occurring Neoplasias and Neoplastic Cells

Previous Serial Number: Same

Principal Investigator: Dr. Paul Arnstein

Other Investigators: Dr. Robert J. Huebner, VCB, VO, DCCP, NCI
Dr. Padman Sarma, VCB, VO, DCCP, NCI
Dr. George Todaro, VLLB, VO, DCCP, NCI
Dr. Stuart Aaronson, VCB, VO, DCCP, NCI

Cooperating Units: Outside NIH:

Dr. E.H. Lennette, California State Department of Public Health, Berkeley, California
Dr. M. Gardner, USC School of Medicine, Cancer Research Laboratory, Los Angeles, California
Dr. R. McAllister, Childrens Hospital, Los Angeles, California
Dr. P. Price, Microbiological Associates, Inc., Bethesda, Maryland
Dr. R. Gilden, Flow Laboratories, Rockville, Maryland
Dr. W. Nelson-Rees, Naval Biological Research Laboratory, Oakland, California
Dr. J. Rhim, Microbiological Associates, Inc., Bethesda, Maryland
Dr. J. Levy, University of California Medical Center, San Francisco, California

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

(1) Design and develop systems for testing anti-cancer vaccines in mice: carry out experiments which will measure response to vaccines prepared from

type C viruses as well as selected other immunogens. (2) Study spontaneous and induced neoplasia with emphasis on in vivo tumor formation and transplantability. (3) Attempt to activate tumor viruses by hetero-transplantation, irradiation and chemical induction (simply or in cocarcinogenesis experiments).

Methods Employed and Major Findings:

Vaccination Experiments: It has been shown that a transplantable virus-negative fibrosarcoma of NIH Swiss mice, N275, will produce a predictable incidence of transplanted tumors in NIH Swiss weanlings with graded doses of tissue culture-grown cells. The tumors are usually progressive and ultimately fatal. Almost 100% tumor incidence can be expected if 5×10^4-10^5 cells are used subcutaneously. The incidence is 50% at 10^3-10^4. Our usual challenge is $5\text{-}7 \times 10^4$ cells subcutaneously. Listed below are cellular vaccines selected for our initial protection tests:

Living cell culture vaccines: (All were given at 10^7 viable cells per mouse subcutaneously)

1. 34489 (Hartley) - suspension line from NIH Swiss X-ray leukemia, non-tumorigenic for NIH Swiss weanlings.
2. NIH 3T3--AKR - NIH Swiss embryo -+ AKR virus (chronically infected live), non-tumorigenic for NIH Swiss weanlings.
3. NZB C152-3105 - NZB lines, chronically infected with xenotrop type C virus. Non-tumorigenic for NIH Swiss weanlings.

Killed cell culture vaccines: (2 doses of 10^7 killed cells each, subcutaneously)

4. N275K - glutaraldehyde-inactivated challenge cells (NIH Sw fibrosarcoma N275).
5. N275 AKRK - fibrosarcoma N275 superinfected with AKR virus and subsequently glutaraldehyde inactivated.
6. NIH 3T3-AKRK - chronically infected fibroblasts inactivated in glutaraldehyde (see #2).
7. 34489K - glutaraldehyde-inactivated Hartley leukemia cells (see #1).

Non-specific immunostimulant: (2 doses, 0.3cc each)

8. CBV - corynebacterium parvum vaccine, glutaraldehyde-inactivated culture, 10% (V/V) packed bacteria.

All groups were challenged 1 month after last dose of vaccine, using 6 x 10^5 N2/75 cells subcutaneously, with appropriate controls, with the following results (a summary of several experiments):

Group	Vaccine	Progressive Tumor Incidence	
Ø	None	12/15	(80%)
1.	NIH 34489 live	6/26	(23%)
2.	NIH 3T3 - AKR live	1/6	(17%)
3.	NZB (C152-3105) live	7/14	(50%)
4.	N275 K	1/4	(25%)
5.	N275 AKRK	4/6	(67%)
6.	NIH 3T3 AKR-K	3/5	(60%)
7.	NIH 34489-K	6/7	(85%)
8.	CBV - K	2/9	(22%)

Non-tumorigenic live cell vaccines of NIH Swiss origin (# 1, # 2) gave significant protection. The NZB cell vaccines were only marginal, whereas killed murine cell vaccines were ineffective, with the possible exception of killed challenge line N275 (# 4). Interestingly, this same line when infected with AKR virus and then used as a killed vaccine failed to protect group 5. It is possible that the AKR virus elicited "blocking" antibody. (Because of the small numbers of mice involved, the tests need to be repeated.) Surprisingly high resistance was exhibited by mice receiving the corynebacterial vaccine (group 8) which was originally expected to be used as a control substance. We have some evidence that the resistance to the sarcoma is cell mediated, most probably T-cell dependent:

(a) We cannot show protection in "nude" mice;
(b) Concentrated lymphotytes from spleen and lymph node of tumor-resistant mice prevent transplantation of tumor cells to normal sucklings. Similar lymphocyte mixtures from non-immune mice do not interfere with the expected tumor formation.

Live and killed murine cellular vaccines and the corynebacterial immunogen are on test also against tumorigenic challenge with 3-methylcholanthrene (3MC). The animals have shown delay of onset of progressive tumors when given live non-tumorigenic leukemia cells (34489, see above) and the corynebacteria; increased tumor incidence and severity was observed in mice receiving killed 34489 cellular vaccine. Current status is summarized below. The animals are still under observation.

Vaccination	Challenge	Progressive tumors (following 3MC) Day 70	Day 100	Day 130
Ø	3MC, 300 mcg	3/6	4/6	6/6
FBS, 2 doses	" "	3/6	6/6	6/6
Corynebacteria, 10% (V/V), killed, 2 doses	" "	1/8	3/8 (s)	6/8
34489 cells, live, 2 doses, 10^7 each	" "	2/10	9/10 (s)	9/10
34489 cells, killed, 2 doses, 10^7 each	" "	8/10	9/9 *	9/9

(s) tumors significantly smaller than in controls.
* tumors significantly larger than in controls.

Athymic "nude" mice are bred and used for transplantation studies permitting greater efficiency and longer maintenance of tumors than in antithymocyte-treated mice. Very slowly growing heterotransplanted tumors result in "no takes" when ATS-treated mice are used, because of reduced ATS efficacy after 1 month. Such tumors have been observed to slowly progress in "nude" athymic mice for over 4 months. In all instances tumors assembled by the neoplastic cultures in the "nude" correspond very closely (histopathologically) to the tumors of origin.

A summary of transplantation of non-murine cell cultures to "nude" mice is summarized below. It should be noted that several of these transplants (e.g., NNu 534, NNu 606, NNu 555, NNu 631, NNu 598 - see Table A) would have probably resulted in no "takes" in the ATS-treated "normal" mice. Cells exhibiting "benign" in vitro morphology almost never produce persistent nodules or tumors - see Table B.

A. NEOPLASTIC APPEARING MONOLAYERS AND SUSPENSION LINES

Experiment	Inoculum	Tumorigenicity	Time span, days
NNu 534	Human leukemia CCRF-CEM (long-term TC suspension line)	++	40-59
NNu 598	Human lymphoma HT1417 (suspension TC)	+ (TC only)	40-50
NNu 541	Human choriocarcinoma JAR (TC p. 178)	++++	15-30

(A, continued)

Experiment	Inoculum	Tumorigenicity	Time span, days
NNu 555	Human sq. cell carcinoma	++	22-40
NNu 571	Human pancreas carcinoma HS766T (TC p. 5)	+++	11-51
NNu 584	Human bladder carcinoma HT1197 (TC p. 23)	++	20-56
NNu 606	Human bladder carcinoma HT1376 (TC p. 12)	++	30-47
NNu 567	Fetal dog thymus 7977, TC p. 123 (spontaneous transformation)	++	12-28
NNu 631	Dog osteosarcoma R20 F12 (radiation-induced) (TC p. 17)	++	44-130
NNu 593	MH Sw leukemia 34489 (suspension line)	++	10-45
NNu 542	Monkey carcinoma CA390I (Rabin) (TC p. 19)	+++	30-70

B. BENIGN APPEARING MONOLAYERS

Experiment	Inoculum	Tumorigenicity
NNu 583	H121 human papilloma (wart) (TC p. 10)	-
NNu 582	H112 human papilloma (wart)	-
NNu 613	H2189 human papilloma (wart) (TC p. 5)	-
NNu 607	Human cord cell H2505 (TC p. 10)	-
LNu 670	Human fibrosarcoma 8387 (TC p. 202)	-
NNu 666	Human embryo K7121 (TC p. 29)	-
LNu 679A	Human embryo kidney (TC p. 22), K948	-
LNu 679B	Human embryo lung (TC p. 22), K948	-
NNu 657	BSC-1 normal monkey (TC p. 184)	-
NNu 654	NBE-3 normal beagle embryo (TC p. 124)	-
NNu 669	Fetal cat Fc79 (TC p. 114)	-
LNu 696	Fischer rat embryo H3305 (TC p. 39)	-

Nude mice have been used to demonstrate a very significant consequence of spontaneous or induced in vitro transformation of cell cultures: it is usually accompanied by ability to propagate as tumor in vivo.

In collaboration with P. Price and J. Rhim, we found that almost invariably when cultures transform from an in vitro "benign" morphology to one which the tissue culture investigator considers "malignant", the pre-transformed cultures prove non-tumorigenic and the transformed highly tumorigenic:

Culture	In vitro rating	In vivo results (nude athymic mice)*	
F364, C57 Bro, p. 53	Benign	No tumors or nodules	**
F1752, NIH Sw, p. 64	Benign	"	**
F6746, C57BL/Cum, p. 59	Benign	Slowly growing tumors	/
F1656, AKR, p. 48	Benign	No tumors or nodules	**
3T3B, NIH Sw, high passage	Benign	"	
3T3Ab, NIH Sw, high passage	Aberrant	Slowly growing tumors	**
H598, BALB/c, p. 53	Malignant	Rapidly growing tumors	**
H607, BALB/c, p. 55	"	"	**
H604, BALB/c, p. 52	"	"	**
H608, BALB/c, p. 52	"	"	**
H603, BALB/c, p. 30	"	"	**
H606, BALB/c, p. 53	"	"	**
HOS (human osteosarcoma)	Pleomorphic	No tumors	**
HOS-KiMSV-transformed	Malignant	Progressive tumors	**
HOS-dimethyl benzanthracine-transformed	Malignant	"	**
HOS-methyl nitrosoguanidine-transformed	Malignant	"	**

* Mice given 5 x 10^6 cells, subcutaneously.
** Predicted behavior.
/ Contrary to prediction.

It is readily apparent that only culture #3 (F6746) produced in vivo results which were contrary to expectations based on in vitro morphology.

Isolation of type C viral strains and their control in nude mice: Transplantation of heterospecific tumor cells to hybrid nude (NIH Swiss and BALB/c) mice has resulted in the isolation of three kinds of type C virus from this mouse--a B-tropic, an N-tropic, and a xenotropic. Isolations were identified in collaboration with P. Price.

The hybrid "nude" mice are now continuously inbred to C57/L stock in order to furnish "cleaner" nude athymic stock. We now have "nudes" which are 7/8 Leaden geneology; these are currently used for special studies because of short supply. The colony is expanding satisfactorily so they may become available for all transplantation.

Significance to Biomedical Research and the Program of the Institute:

Vaccine experiments using mice should be the most basic first step in selecting appropriate anti-tumor immunogens. Provided dosages, challenge methods and strains of mice are adequately sampled and necessary adjustments made, efficient evaluations, narrowing the choices of promising vaccines to a few types, should be obtained. Such "zeroing in" on preparations of maximum protection against several challenge methods would conserve time and funds: only the best materials would have to be re-tested in other more expensive and difficult animal models.

Transplantation as a method of (a) inducing tumor viruses and (b) confirming *in vitro* malignant transformation offers still unexplored possibilities.

Proposed Course:

(a) Amplify immunization trials;
(b) Continue tumorigenicity and transplantation experiments on selected materials, using nude athymic mice;
(c) Continue attempts to isolate type C viruses from neoplasms.

Honors and Awards:

None

Publications:

Arnstein, P., Levy, J.A., Oshiro, L.S., Price, P.J., Suk, W. and Lennette, E.H.: Recovery of murine xenotropic type C virus from C57L mice. J. Natl. Cancer Inst. 53: 1787-1792, 1974.

Stephenson, J.R., Aaronson, S.A., Arnstein, P., Huebner, R.J., and Tronick, S.R.: Demonstration of two immunologically distinct xenotropic type C RNA viruses of mouse cells. Virology 61: 56-63, 1974.

Serial No. Z01-CP-04933-01-VC

1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on expression of the viral genome in murine sarcoma virus transformed nonproducer cells

Previous Serial Number: NONE

Principal Investigator: Dr. William I. Bensinger

Other Investigators: Dr. Stuart A. Aaronson
Dr. John R. Stephenson
Dr. Steven R. Tronick

Cooperating Units: Inside NIH

Dr. Robert J. Huebner, VCB, NCI

Outside NIH

None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. Study genetic factors required for expression of transformation by tumor viruses by obtaining and analyzing morphologic revertants of MSV nonproducer cells which contain normal sarcoma viruses.

2. Isolation and characterization of murine sarcoma virus mutants temperature sensitive for phenotypic expression of the transformed state.

3. Determine the role of murine leukemia virus antigens in stimulating host immunity to experimental solid tumors.

4. Study in vivo host regulation of genetic expression of a horizontally transmitted leukemia virus.

Methods Employed:

Standard tissue culture techniques for in vitro work. Animal facilities for several hundred mice and rats and standard in vivo immunologic techniques.

Major Findings:

1. Improved methods for isolation and selection of MSV nonproducer cell revertants have led to the isolation of several additional revertant clones.

2. Methods are being developed for selection of temperature sensitive murine sarcoma virus mutants.

Significance to Biomedical Research and to the Program of the Institute:

Analysis of the viral and cellular functions involved in expression of MSV transformation should increase our knowledge of the mechanism of action of this oncogenic virus. Studies of the immunologic effects of type C virus production on tumor cell growth may lead to methods by which to prevent and/or control virus-induced tumors.

Proposed Course:

1. Genetic experiments with MSV nonproducer flat revertants to establish the number of genetic factors required for transformation.

2. Immunotherapy of solid experimental tumors in rats.

Honors and Awards:

None

Publications:

None

Serial No. ZO1-CP-04940-08-VC
1. Viral Carcinogenesis Branch,
   OADVO, Division of Cancer
   Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Viruses in experimental oncogenesis and human cancer

Previous Serial Number: SAME

Principal Investigator: Dr. Stuart A. Aaronson

Other Investigators: Dr. John R. Stephenson
Dr. Steven R. Tronick
Dr. Mariano Barbacid
Dr. William I. Bensinger
Dr. Shigeo Hino
Dr. Cirilo D. Cabradilla

Cooperating Units: Inside NIH

Dr. Robert J. Huebner, VCB, NCI
Dr. Malcolm Martin, LBV, NIAID
Dr. Kenneth K. Takemoto, LVD, NIAID
Dr. Paul Arnstein, VCB, NCI
Dr. Tadao A. Aoki, VLLB, NCI
Dr. Gary J. Kelloff, VCB, NCI
Dr. Frank H. Portugal, VCB, NCI

Outside NIH

Dr. Roger E. Wilsnack, Huntingdon Research Laboratories, Baltimore, Maryland
Dr. Robert Peters, Microbiological Associates, Inc., Walkersville, Maryland
Dr. Murray Gardner, University of Southern California, Los Angeles, California
Dr. Eugene J. Smith, U. S. Department of Agriculture, Beltsville, Maryland
Dr. Lyman B. Crittenden, U. S. Department of Agriculture, Beltsville, Maryland
Dr. Michael Hanna, Frederick Cancer Research Center, Frederick, Maryland
Dr. Frank Dixon, Scripps Clinic and Research Foundation, La Jolla, California

Dr. Henry Kaplan, Stanford University,
Stanford, California
Dr. David G. Baltimore, Massachusetts Institute of
Technology, Cambridge, Massachusetts
Dr. Raymond V. Gilden, Flow Laboratories,
Rockville, Maryland
Dr. Thomas G. Kawakami, The Salk Institute,
San Diego, California
Dr. William C. Moloney, Peter Bent Brigham Hospital,
Boston, Massachusetts
Dr. Victor Kalnins, University of Toronto, Canada
Dr. Tak W. Mak, University of Toronto, Canada
Dr. Joel S. Greenberger, Joint Center for Radiation
Therapy, Boston, Massachusetts

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To study the mechanisms of action of RNA and DNA-containing tumor viruses.

2. To apply knowledge gained from experimental systems to search for a viral etiology to human neoplasia and to develop rational approaches to prevention and treatment of human tumors.

Methods Employed:

Standard and developmental techniques in virology, cell biology, immunology, and biochemistry.

Major Findings:

1. Cells of different inbred mouse strains have been shown to contain different numbers and biologic classes of naturally-integrated type C RNA viruses. Three classes of endogenous viruses are distinguishable in their host ranges and serologic properties as well as in the type-specific determinants of their structural polypeptides. Halogenated pyrimidines induce two viruses from BALB/c mouse cells as well as from cells of many other inbred strains. In contrast, inhibitors of protein synthesis specifically activate one of these viruses. A third class of virus, whose viral polypeptides are expressed at high levels in many strains in the absence of virus release, is not inducible by either drug. These findings demonstrate that different endogenous viruses are specifically regulated by the same cell.

2. Cycloheximide and iododeoxyuridine were shown to induce the same endogenous virus efficiently from cultures in exponential growth but were inactive on cells in stationary phase. However, cells were found to be maximally sensitive to the actions of the two drugs at different times within the cell cycle. Further, virus induction in response to each drug was differentially inhibited under conditions of simultaneous cell exposure to inhibitors of DNA or RNA synthesis. These results support the concept that inhibitors of protein synthesis and halogenated pyrimidines act by different mechanisms to induce release of the same type C virus.

3. The mechanism of type C virus activation by inhibitors of protein synthesis was investigated. Virus activation in response to a representative translational inhibitor, cycloheximide, was transient, with virus release primarily occurring within the first 12 to 24 hours following drug exposure. Analysis of virus-specific RNA in cells by molecular hybridization revealed an absolute increase in viral RNA concentration in cycloheximide-treated cells. This was blocked by simultaneous exposure of the cells to actinomycin D. Inhibition of cellular RNA synthesis during but not subsequent to cycloheximide exposure prevented virus activation. These findings demonstrate that virus induction by cycloheximide requires de novo RNA synthesis during but not after drug exposure and suggests that the required RNA species may be that of the virus itself. These findings are consistent with the hypothesis that translational inhibitors prevent synthesis of a labile protein whose normal action is to inhibit viral RNA transcription or to cause degradation of viral RNA.

4. The regulatory locus, Fv-1, has been shown to influence the expression of the class of endogenous virus that is NIH-tropic in host range. The presence of a second class of virus, xenotropic in host range and by itself unaffected by Fv-1, was shown to permit the first virus to partially overcome the restriction at Fv-1 in nonpermissive cells. These findings provide evidence that the interaction between different endogenous viruses plays the role in their expression.

5. A class of xenotropic virus, which is common to mice of NIH Swiss and NZB strains, is spontaneously released at high level by NZB embryo cells while its production by NIH Swiss embryo cells is below detectable levels. Quantitative analysis of the chronic level of virus release by embryo cell lines established in tissue culture from genetic crosses of NIH and NZB strains led to demonstration of the existence of a partially dominant gene influencing the release of this virus. The evidence indicates that this gene may act to provide a factor necessary for expression of this class of endogenous virus.

6. The oncogenic potential of different classes of endogenous type C RNA viruses has been investigated. Both C58 and BALB/c cells have been shown to contain NIH-tropic endogenous viruses. The C58-induced virus is around 10-fold more infectious per physical particle than the NIH-tropic virus of BALB/c cells. Inoculation of these viruses into newborn NIH Swiss mice led to the development of diseases associated with splenomegaly and lymphadenopathy at similar frequency in each group. The disease induced by C58-MuLV

was histopathologically diagnosed as lymphoblastic leukemia and was highly malignant following transplantation into newborn mice. The histopathologic appearance of spleens from BALB/c virus-infected animals was distinguishable, demonstrating instead myeloid metaplasia or myelogenous leukemia. These findings indicate that different endogenous mouse type C viruses can induce distinct diseases in the same mouse strain. Furthermore, they implicate the NIH-tropic virus endogenous to C58 cells as a major factor in the development of lymphoblastic leukemia that occurs at high frequency in that strain.

7. A genetic approach to the study of RNA tumor viruses has led to the isolation of a large number of conditional lethal mutants of the Rauscher and Kirsten strains of MuLV. These have been characterized physiologically into three classes, all of which are defective in replication steps subsequent to absorption and penetration. The first class contains mutants that enter the cell but fail to integrate at the nonpermissive temperature; the second class produces viral antigen but not completed virus at the nonpermissive temperature. However, those virions are noninfectious at either permissive or nonpermissive temperatures on subsequent infection. The first class of mutants has been shown to be defective in providing functions necessary for integration of murine sarcoma virus at the nonpermissive temperature. Further, one of these mutants contains a reverse transcriptase that is temperature-sensitive. Genetic recombination between different pairs of mutants has also been demonstrated. The recombinants were shown to grow equally well at the permissive and nonpermissive temperatures and to have serologic characteristics of the wild-type parental virus.

8. Morphologic revertants of sarcoma virus transformed nonproducer cells have been isolated. These clonal lines show normal cell morphology but still contain the sarcoma viral genome. A class of morphologic revertants that is as susceptible as the normal cell to exogenous sarcoma virus infection has been investigated. These revertants are shown to contain levels of sarcoma viral RNA, quantitatively and qualitatively, indistinguishable from that present in the parental transformed clone. Further, following rescue with helper leukemia virus, these cells release low levels of wild-type transforming virus and a large excess of transformation-defective sarcoma virus as measured by molecular hybridization. The defective viruses can be transmitted to new cells in the absence of morphologic alteration. These results provide strong evidence that the revertants contain mutant sarcoma viruses defective in some transforming function. These revertants should be useful in complementation studies to determine the number of functions involved in sarcoma virus transformation. Sarcoma virus mutants obtained in this manner appear to be the first obtained by spontaneous or chemically-induced genetic alteration of a stably integrated virus in eukaryotic cells.

9. The MSV nonproducer cell has been of particular interest because it provides a model system with many apparent similarities to the human tumor cell. Nonproducer cells lack detectable transplantation antigens, in contrast to RNA virus-producing transformed cells or DNA transformed cells, both of which are highly antigenic. Although these cells lack a transplantation antigen, they do contain MSV-associated cell surface antigens. Analysis

of the cell surface antigens of mouse sarcoma virus and woolly sarcoma virus transformed cells has revealed the presence of several sarcoma virus-related antigens. One of these is shared by cells transformed by both the mouse and primate sarcoma virus. These antigens provide the only known sarcoma virus-related antigenic markers of sarcoma virus presence within the transformed cell.

10. The cell surfaces of MSV nonproducer BALB/3T3 cells and the nontransformed parental BALB/3T3 clone have been analyzed biochemically. The ganglioside composition of the MSV nonproducer subclone was drastically altered compared to the nontransformed parental clone. The transformed clone was unable to synthesize the mono- and disialogangliosides, GM1 and GD1A, due to the complete absence of a specific galactosyl transferase. The lack of this enzyme activity was established by sensitive radiochemical and enzymological techniques in vivo and in vitro. These biochemical findings indicate that the cell surface of the MSV-transformed cell is markedly altered compared to that of the normal cell.

11. Very sensitive and highly specific immunologic assays have been developed for several polypeptides including p30, p12, and the reverse transcriptase of a large number of mammalian and avian type C RNA viruses. Type-specific immunoassays have proven of great use in distinguishing different strains of the same species of virus. These assays have also been very useful in the identification of new type C virus isolates of potential human origin. In type-specific immunoassays for the p12 polypeptides of gibbon ape and woolly monkey type C RNA viruses, two new type C viruses isolated from gibbons in another colony characterized by high incidence of hemaplatic neoplasia have been shown to be immunologically distinguishable from the original gibbon ape virus. These and other immunoassays for low molecular weight polypeptides of primate virus isolates should be of importance in studying the natural history of these viruses and their relationship to primate tumors.

12. The immunologic response of different strains of mice to the natural expression of their endogenous viruses has been examined. High titers of neutralizing antibodies have been detected in a class of endogenous virus, xenotropic in host range. In contrast, the same sera failed to react against NIH-tropic endogenous viruses also known to exist in cells of the same strains. These findings demonstrate the ability of the mouse to immunologically respond to a class of type C viruses, whose mode of transmission is similar to that of cellular genes.

13. Neutralizing activity in normal mouse serum to xenotropic endogenous virus was found to be associated with the 19S but not the 7S immunoglobulin fraction. While the role of the autogenous humoral immune response in naturally-occurring diseases is not yet known, it may provide a systemic mode of regulation of endogenous type C virus expression in vivo.

14. Biochemical and immunologic techniques that have been developed for the investigation of the partial expression of known type C RNA viruses in model

systems have been applied to the detection of viral information in human cells. A large number of human tumors have been processed, and several new lines of human tumor cells have been established in culture. These are being extensively investigated for evidence of tumor virus expression using techniques developed from work in model systems.

Significance to Biomedical Research and the Program of the Institute:

The systems that are being intensively investigated have provided a much better understanding of the biology and biochemistry of viral transformation. It is felt that a clear understanding of these phenomena will significantly speed progress in the search for a viral etiology of human cancer.

Proposed Course:

To continue research already in progress in the following major areas: (1) mechanisms of action of murine sarcoma and murine leukemia viruses; (2) induction and regulation of type C viruses in virus-negative cells; (3) determination of the role of viruses in human neoplasia; and (4) to develop new research areas in model systems that pertain to human disease.

Honors and Awards:

None

Publications:

Stephenson, J. R. and Aaronson, S. A.: Temperature-sensitive mutants of murine leukemia virus. III. Mutants defective in helper functions for sarcoma virus fixation. Virology 58: 294-297, 1974.

Aaronson, S. A. and Stephenson, J. R.: Widespread natural occurrence of high-titered neutralizing antibodies to a specific class of endogenous mouse type C virus. Proc. Nat. Acad. Sci. U.S.A. 71: 1957-1961, 1974.

Tronick, S. R., Stephenson, J. R., and Aaronson, S. A.: Immunological properties of two polypeptides of Mason-Pfizer monkey virus. J. Virol. 14: 125-132, 1974.

Greenberger, J. S., Anderson, G. R., and Aaronson, S. A.: Transformation-defective virus mutants in a class of morphologic revertants of sarcoma virus-transformed nonproducer cells. Cell 2: 279-286, 1974.

Fishman, P. H., Brady, R. O., Bradley, R. M., Aaronson, S. A., and Todaro, G. J.: Absence of a specific ganglioside galactosyltransferase in murine sarcoma virus-transformed mouse cells. Proc. Nat. Acad. Sci. U.S.A. 71: 298-301, 1974.

Stephenson, J. R., Anderson, G. R., Tronick, S. R., and Aaronson, S. A.: Evidence for genetic recombination between endogenous and exogenous mouse

RNA type C viruses. Cell 2: 87-94, 1974.

Stephenson, J. R., Tronick, S. R., and Aaronson, S. A.: Temperature-sensitive mutants of murine leukemia virus. IV. Further physiologic characterization and evidence for genetic recombination. J. Virol. 14: 918-923, 1974.

Stephenson, J. R., Aaronson, S. A., Arnstein, P., Huebner, R. J., and Tronick, S. R.: Demonstration of two immunologically distinct xenotropic RNA type C viruses of mouse cells. Virology 61: 56-63, 1974.

Aaronson, S. A., Stephenson, J. R., and Greenberger, J. S.: Cellular replication and the persistence of inducible RNA type C viruses. J. Virol. 13: 1404-1407, 1974.

Greenberger, J. S., Stephenson, J. R., Aoki, T., and Aaronson, S. A.: Cell surface antigens of murine sarcoma virus transformed nonproducer cells: Further evidence for lack of transplantation immunity. Int. J. Cancer 14: 145-152, 1974.

Aoki, T., Stephenson, J. R., Aaronson, S. A., and Hsu, K. C.: Surface antigens of mammalian sarcoma virus-transformed nonproducer cells. Proc. Nat. Acad. Sci. U.S.A. 71: 3445-3449, 1974.

Stephenson, J. R., Crow, J. D., and Aaronson, S. A.: Differential activation of biologically distinguishable endogenous mouse type C RNA viruses. Interactions with host cell regulatory factors. Virology 61: 411-419, 1974.

Aaronson, S. A., Anderson, G. R., Dunn, C. Y., and Robbins, K. C.: Induction of type C RNA virus by cycloheximide: Increased expression of virus-specific RNA. Proc. Nat. Acad. Sci. U.S.A. 70: 3941-3945, 1974.

Lee, J. C., Hanna, Jr., M. G., Ihle, J. N., and Aaronson, S. A.: Autogenous immunity to endogenous RNA tumor virus: Differential reactivities of immunoglobins M and G to virus envelope antigens. J. Virol. 14: 773-781, 1974.

Aaronson, S. A. and Stephenson, J. R.: Cellular regulation of biologically distinguishable endogenous mouse type-C RNA viruses. In Sercarz, E., Williamson, A. and Fox, C. (Eds.): Proc. Third ICN-UCLA Symposium on Molecular Biology. New York, Academic Press, 1974, pp. 441-445.

Stephenson, J. R., Tronick, S. R., and Aaronson, S. A.: Isolation from BALB/c mouse cells of a structural polypeptide of a third endogenous type C virus class. Cell 3: 347-353, 1974.

Stephenson, J. R. and Aaronson, S. A.: Demonstration of a genetic factor influencing spontaneous release of a xenotropic virus of mouse cells. Proc. Nat. Acad. Sci. U.S.A. 71: 4925-4929, 1974.

Tronick, S. R., Stephenson, J. R., Aaronson, S. A., and Kawakami, T. G.: Antigenic characterization of type C RNA virus isolates of gibbon apes. J. Virol. 15: 115-120, 1974.

Greenberger, J. S. and Aaronson, S. A.: Cycloheximide induction of xenotropic type C virus from synchronized mouse cells: Metabolic requirements for virus activation. J. Virol. 15: 64-70, 1975.

Greenberger, J. S., Stephenson, J. R., Moloney, W. C., and Aaronson, S. A.: Different hematologic diseases induced by type C viruses chemically activated from embryo cells of different mouse strains. Cancer Res. 35: 245-252, 1975.

Aaronson, S. A. and Stephenson, J. R.: Differential cellular regulation of three distinct classes of type C RNA viruses endogenous to mouse cells. Cold Spring Harbor Symposium on Quantitative Biology, 39, 1975, pp. 1129-1137.

Aaronson, S. A. and Stephenson, J. R.: Viruses and the etiology of cancer. In Drew, W. L. (Ed.): Clinical Concepts in Virology. New York, Medcom Press, (In press).

Aaronson, S. A. and Schlom, J.: The search for RNA tumor viruses in human cancer. In Ariel, I. (Ed.): Progress in Clinical Cancer. New York, Grune and Stratton, Inc., (In press).

Greenberger, J. S., Rosenthal, D. S., Aaronson, S. A., and Moloney, W. C.: Acute myelogenous leukemia of the Wistar/Furth rat: Establishment of a continuous tissue culture line producing lysozyme in vitro and in vivo. Blood, (In press).

Tronick, S. R., Stephenson, J. R., Verma, I. M., and Aaronson, S. A.: A mutant of murine leukemia virus with a thermolabile reverse transcriptase. In Fox, C. F. (Ed.): Proc. ICN-UCLA Conferences in Molecular and Cellular Biology, (In press).

Serial No. ZO1-CP-04941-03-VC
1. Viral Carcinogenesis Branch,
   OADVO, Division of Cancer
   Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical and immunological studies of the proteins of RNA reverse transcriptase-containing viruses

Previous Serial Number: SAME

Principal Investigator: Dr. Steven R. Tronick

Other Investigators:
Dr. Stuart A. Aaronson
Dr. John R. Stephenson
Dr. Shigeo Hino
Dr. Mariano Barbacid
Dr. Cirilo D. Cabradilla
Dr. William I. Bensinger

Cooperating Units:

Inside NIH

Dr. Robert J. Huebner, VCB, NCI
Dr. Frank H. Portugal, VCB, NCI

Outside NIH

Dr. Roger E. Wilsnack, Huntingdon Research Laboratories, Baltimore, Maryland
Dr. Thomas G. Kawakami, University of California, Davis, California
Dr. David G. Baltimore, Massachusetts Institutes of Technology, Cambridge, Massachusetts
Dr. Inder M. Verma, The Salk Institute, San Diego, California

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To purify the proteins of RNA tumor viruses.

2. To continue development of sensitive and specific assays for viral proteins.

3. To determine the functions of virion proteins, especially in regard to their possible role in the malignant transformation of virus-infected cells.

4. To biochemically characterize temperature sensitive mutants of murine leukemia virus in order to understand the mechanism of replication of mammalian type C viruses.

5. To determine the involvement of reverse-transcriptase-containing viruses in cancers of primates.

Methods Employed:

Standard biochemical techniques for protein isolation and analysis; assays for enzymes of nucleic acid synthesis and degradation; radioimmunoassays for qualitative and quantitative characterization of tumor viruses and their proteins.

Major Findings:

1. Using radioimmunoassays for the woolly monkey type C viral p12, an antigenically related polypeptide was isolated from the gibbon ape type C virus. The molecular weight of this polypeptide was also found to be 12,000 daltons and is designated p12. Using radioimmunoassays for the gibbon ape and woolly monkey viral p12 polypeptides, independent isolates of gibbon type C viruses were antigenically characterized. The prototype gibbon type C virus, isolated from animals at the University of California, San Francisco, Medical Center, was readily differentiated from type C viruses isolated from gibbons in a colony in the SEATO Laboratory, Bangkok, Thailand. Each gibbon virus isolate was antigenically distinct from the woolly monkey type C virus.

2. Two polypeptides, p26 and p15, were isolated and purified from the Mason-Pfizer monkey virus (MP-MV). Radioimmunoassays developed for the quantitation of these polypeptides were 10-100 times more sensitive than previously available immunological tests, and were also highly specific for the detection of the respective polypeptide and MP-MV virions.

3. The heat inactivation kinetics of the reverse transcriptase activity of temperature sensitive mutants of murine leukemia virus revealed that one early replication mutant, ts 29, possesses a thermolabile enzyme. This provides direct evidence for the involvement of the reverse transcriptase in

leukemia virus replication and also confirms that this enzyme is a viral gene product. Ts 29 and other early mutants are unable to provide helper functions for the defective murine sarcoma virus (MSV) genome at the nonpermissive temperature. However, only the ts 29 polymerase was found to be thermolabile. This result suggests that other leukemia virus gene products are required in order for the MSV genome to transform cells.

Significance to Biomedical Research and the Program of the Institute:

The availability of highly specific and sensitive immunological assays for purified oncorna virus polypeptides has become increasingly important in view of recent reports of Mason-Pfizer-like and type C viruses in human tumor cells. These immunoassays will be of value in studying the epidemiology of the diseases with which these viruses are associated. Furthermore, the type-specific p12 assays are useful in determining the relatedness of virus isolates from human cells to known RNA-containing viruses.

Assays for viral structural proteins and enzymes also aid in the study of the mode of replication and cellular regulation of type C viruses, and should help in understanding the molecular basis for malignant transformation of mammalian cells by these viruses.

Proposed Course:

The present studies on proteins of primate type C viruses will be continued and will be extended to new isolates of virus from primates. Temperature sensitive leukemia virus mutants will be studied in greater detail in order to determine the nature of functions in addition to the reverse transcriptase that are involved in the replication of these viruses.

Honors and Awards:

None

Publications:

Tronick, S. R., Stephenson, J. R., and Aaronson, S. A.: Comparative immunological studies of RNA C type viruses: Radioimmunoassay for a low molecular weight polypeptide of woolly monkey leukemia virus. Virology 57: 347-356, 1974.

Stephenson, J. R., Aaronson, S. A., Arnstein, P., Huebner, R. J., and Tronick, S. R.: Demonstration of two immunologically distinct xenotropic RNA type C viruses of mouse cells. Virology 61: 244-248, 1974.

Tronick, S. R., Stephenson, J. R., and Aaronson, S. A.: Immunological properties of two polypeptides of Mason-Pfizer monkey virus. J. Virology 14: 125-132, 1974.

Stephenson, J. R., Anderson, G. R., Tronick, S. R., and Aaronson, S. A.: Evidence for genetic recombination between endogenous and exogenous mouse RNA type C viruses. Cell 2: 87-94, 1974.

Stephenson, J. R., Tronick, S. R., and Aaronson, S. A.: Temperature sensitive mutants of murine leukemia virus. IV. Further physiologic characterization and evidence for genetic recombination. J. Virology 14: 918-923, 1974.

Stephenson, J. R., Tronick, S. R., and Aaronson, S. A.: Isolation from BALB/c mouse cells of a structural polypeptide of a third endogenous type C virus class. Cell 3: 347-353, 1974.

Tronick, S. R., Stephenson, J. R., Aaronson, S. A., and Kawakami, T. G.: Antigenic characterization of RNA type C viruses isolated from gibbon apes. J. Virology 15: 115-120, 1975.

Tronick, S. R., Stephenson, J. R., Verma, I. M., and Aaronson, S. A.: A mutant of murine leukemia virus with a thermolabile reverse transcriptase. In Fox, C. F. (Ed.): Proc. ICN-UCLA Conferences in Molecular and Cellular Biology, (In press).

Serial No. Z01-CP-04942-05-VC

1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular regulation of endogenous type C RNA tumor viruses of mammalian cells

Previous Serial Number: SAME

Principal Investigator: Dr. John R. Stephenson

Other Investigators: Dr. Stuart A. Aaronson
Dr. Steven R. Tronick
Dr. Shigeo Hino
Dr. Cirilo D. Cabradilla
Dr. Mariano Barbacid
Dr. William I. Bensinger

Cooperating Units: Inside NIH

Dr. Robert J. Huebner, VCB, NCI
Dr. Tadao A. Aoki, VLLB, NCI
Dr. Gary J. Kelloff, VCB, NCI
Dr. Frank H. Portugal, VCB, NCI

Outside NIH

Dr. Roger E. Wilsnack, Huntingdon Research Laboratories, Baltimore, Maryland
Dr. Victor Kalnins, University of Toronto, Canada
Dr. Thomas G. Kawakami, University of California, Davis, California
Dr. Inder M. Verma, University of California, San Diego, California
Dr. Eugene J. Smith, U. S. Department of Agriculture, Beltsville, Maryland
Dr. Tak W. Mak, University of Toronto, Canada
Dr. Lyman B. Crittenden, U. S. Department of Agriculture, Beltsville, Maryland
Dr. Raymond V. Gilden, Flow Laboratories, Rockville, Maryland
Dr. William C. Moloney, Peter Bent Brigham Hospital, Boston, Massachusetts

Dr. Joel S. Greenberger, Joint Center for Radiation Therapy, Boston, Massachusetts
Dr. Konrad C. Hsu, Columbia University Medical Center, New York, New York

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To study the mechanisms of replication and transformation by type C RNA viruses, investigate cellular regulatory controls influencing expression of endogenous type C viruses and determine the role of these viruses in human neoplasia.

Methods Employed:

1. Standard tissue culture procedures, including XC plaque assays for leukemia virus and focus forming assays for sarcoma virus.

2. Biochemical and immunologic techniques for isolation and analysis of viral structural proteins: radioimmunoassays for proteins of different classes of mammalian type C RNA viruses.

Major Findings:

1. The in vivo biologic functions of the endogenous N-tropic viruses of C58 and BALB/c cells have been examined. Inoculation of either virus into newborn NIH Swiss mice led to development of diseases associated with splenomegaly and lymphadenopathy at similar frequency. While the disease induced by C58-MuLV was histopathologically diagnosed as lymphoblastic leukemia, the disease induced by the endogenous N-tropic virus of BALB/c mouse cells resembled more closely myeloid metaplasia or myelogenous leukemia. These findings indicate that different endogenous mouse type C viruses can induce distinct diseases in the same mouse strain.

2. A 12,000 molecular weight type C viral polypeptide, p12, has been isolated from BALB/c mouse cells. This polypeptide has been shown to be immunologically distinct from the p12 antigens of previously described N-tropic and xenotropic endogenous viruses of BALB/c cells. It is, however, indistinguishable from a viral antigen expressed in NIH Swiss mouse cells and present in type C viruses isolated from NIH Swiss mice. These findings provide evidence for the existence of genetic information for three distinguishable endogenous viruses within cells of the BALB/c strain.

3. Two classes of chemical inducers, halogenated pyrimidines and inhibitors of protein synthesis, have been shown to differentially affect expression of endogenous viruses containing each of three distinguishable p12 antigens. These findings provide evidence that regulatory factors, specific for each class of endogenous virus, exist within the cell.

4. Cellular regulation of an endogenous xenotropic virus of mouse cells has been examined. The immunologic type-specificity of the p12 antigen of a xenotropic virus spontaneously released at high level from NZB mouse cells has been analyzed by radioimmunoassay and found to be indistinguishable from that of the corresponding polypeptide of the endogenous xenotropic virus of NIH Swiss cells. Quantitative analysis of the chronic level of virus release by individual embryo cell lines established in tissue culture from genetic crosses of NIH and NZB strains has demonstrated the existence of a partially dominant gene influencing expression of this class of endogenous type C virus.

5. The kinetics of chemical activation of different classes of endogenous mouse type C viruses have been studied utilizing MSV nonproducer clones derived from mouse embryo cells with genetically defined virologic characteristics. By this approach the kinetics of activation of two endogenous viruses of BALB/c mouse cells have been shown to be very different. Further, a cellular locus, Fv-1, has been shown to influence the expression of one virus. The presence of a second virus, by itself unaffected by Fv-1, permits the first virus to partially overcome the restriction at Fv-1 in nonpermissive cells. These results provide evidence that the interaction between different endogenous viruses plays a role in their expression.

6. Several previously described temperature-sensitive (ts) mutants of the Rauscher strain of murine leukemia virus representing three distinct physiologic groups have been further characterized. Three R-MuLV ts mutants previously found to be defective in early replication steps have been shown to be ts in post-penetration helper functions which MuLV provides for fixation of transformation by murine sarcoma virus (MSV). The heat inactivation kinetics of the reverse transcriptase enzymes of each of these three ts mutants have been examined. One mutant, ts 29, has been found to possess a thermolabile polymerase. These findings provide the first direct evidence that the reverse transcriptase of mammalian type C viruses is viral-coded and indicate that the MuLV reverse transcriptase is required both for leukemia virus replication and for initiation of transformation by MSV. Genetic recombination between several R-MuLV ts mutants including those defective in both early and late replication steps has been demonstrated. Several representative genetic recombinants have been isolated and shown to replicate as well as wild-type virus at both the permissive and nonpermissive temperatures.

7. Highly type-specific radioimmunoassays have been developed for the 12,000 molecular weight, p12, structural polypeptides of the woolly monkey and gibbon ape type C viruses. Using these assays, several independent isolates

of gibbon ape type C viruses have been antigenically characterized and shown to be distinguishable from the woolly monkey type C virus. In addition, the prototype gibbon ape type C virus, isolated from animals at the University of California, San Francisco, Medical Center, was readily differentiated from type C virus isolates from gibbon apes in a colony at the SEATO Laboratory, Bangkok, Thailand.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the genetic regulatory factors influencing expression of endogenous viruses of mouse cells should provide methods by which to establish whether human cells contain endogenous type C viruses and to determine whether such viruses are involved in the etiology of natural tumors. The availability of highly sensitive and specific radioimmunoassays utilizing purified type C viral polypeptides will be of value for characterization of new type C RNA virus isolates including those of potential human origin.

Proposed Course:

To further examine cellular regulatory controls influencing expression of biologically distinguishable endogenous type C viruses of mammalian cells and to investigate the involvement of type C RNA viruses in natural tumors of different mammalian species.

Honors and Awards:

None

Publications:

Stephenson, J. R. and Aaronson, S. A.: Genetics of endogenous mouse C type viruses. In Ito, Y. and Dutcher, R. M. (Eds.): Proc. Sixth International Symposium on Comparative Leukemia Research. Tokyo, Japan, University of Tokyo Press, 1974, pp. 593-594.

Aaronson, S. A. and Stephenson, J. R.: Widespread natural occurrence of high-titered neutralizing antibodies to a specific class of endogenous mouse C type virus. Proc. Nat. Acad. Sci. U.S.A. 71: 1957-1961, 1974.

Stephenson, J. R. and Aaronson, S. A.: Temperature sensitive mutants of murine leukemia virus. III. Mutants defective in helper functions for sarcoma virus fixation. Virology 58: 294-297, 1974.

Tronick, S. R., Stephenson, J. R., and Aaronson, S. A.: Comparative immunological studies of RNA C type viruses: Radioimmunoassay for a low molecular weight polypeptide of woolly monkey leukemia virus. Virology 57: 347-356, 1974.

Tronick, S. R., Stephenson, J. R., and Aaronson, S. A.: Immunological properties of two polypeptides of Mason-Pfizer monkey virus. J. Virol. 14: 125-132, 1974.

Stephenson, J. R., Tronick, S. R., and Aaronson, S. A.: Temperature sensitive mutants of murine leukemia virus. IV. Further physiologic characterization and evidence for genetic recombination. J. Virol. 14: 918-923, 1974.

Aoki, T., Stephenson, J. R., Aaronson, S. A., and Hsu, K. C.: Surface antigens of mammalian sarcoma virus-transformed nonproducer cells. Proc. Nat. Acad. Sci. U.S.A. 71: 3445-3449, 1974.

Greenberger, J. S., Stephenson, J. R., Aoki, T., and Aaronson, S. A.: Cell surface antigens of murine sarcoma virus transformed nonproducer cells: Further evidence for lack of transplantation immunity. Int. J. Cancer 14: 145-152, 1974.

Stephenson, J. R., Aaronson, S. A., Arnstein, P., Huebner, R. J., and Tronick, S. R.: Demonstration of two immunologically distinct xenotropic RNA type C viruses of mouse cells. Virology 61: 244-248, 1974.

Aaronson, S. A., Stephenson, J. R., and Greenberger, J. S.: Cellular replication and persistence of inducible RNA type C viruses. J. Virol. 13: 1404-1407, 1974.

Aaronson, S. A. and Stephenson, J. R.: Cellular regulation of biologically distinguishable endogenous mouse type C RNA viruses. In Sercarz, E., Williamson, A., and Fox, C. (Eds.): Proc. Third ICN-UCLA Symposium on Molecular Biology. New York, Academic Press, 1974, pp. 441-445.

Stephenson, J. R., Crow, J. D., and Aaronson, S. A.: Differential activation of biologically distinguishable endogenous mouse type C RNA viruses: Interactions with host cell regulatory factors. Virology 61: 411-419, 1974.

Stephenson, J. R., Tronick, S. R., and Aaronson, S. A.: Isolation from BALB/c mouse cells of a structural polypeptide of a third endogenous type C virus. Cell 3: 347-353, 1974.

Stephenson, J. R., Anderson, G. R., Tronick, S. R., and Aaronson, S. A.: Evidence for genetic recombination between endogenous and exogenous mouse RNA type C viruses. Cell 2: 87-94, 1974.

Stephenson, J. R. and Aaronson, S. A.: Demonstration of a genetic factor influencing spontaneous release of a xenotropic virus of mouse cells. Proc. Nat. Acad. Sci. U.S.A. 71: 4925-4929, 1974.

Greenberger, J. S., Stephenson, J. R., Moloney, W. C., and Aaronson, S. A.: Different hematologic diseases induced by type C viruses chemically activated from embryo cells of different mouse strains. Cancer Res. 35: 245-252, 1975.

Tronick, S. R., Stephenson, J. R., Aaronson, S. A., and Kawakami, T. G.: Antigenic characterization of type C RNA virus isolates of gibbon apes. J. Virol. 15: 115-120, 1975.

Aaronson, S. A. and Stephenson, J. R.: Differential cellular regulation of three distinct classes of type C RNA viruses endogenous to mouse cells. Cold Spring Harbor Symposium on Quantitative Biology, 39, 1129-1137, 1975.

Aaronson, S. A. and Stephenson, J. R.: Viruses and the etiology of cancer. In Drew, W. L. (Ed.): Clinical Concepts in Virology. New York, Medcom Press, (In press).

Tronick, S. R., Stephenson, J. R., Verma, I. M., and Aaronson, S. A.: A mutant of murine leukemia virus with a thermolabile reverse transcriptase. In Fox, C. F. (Ed.): Proc. ICN-UCLA Conferences in Molecular and Cellular Biology, (In press).

Serial No. Z01-CP-04943-01-VC

1. Viral Carcinogenesis Branch, OADVO, Division of Cancer Cause and Prevention
2. Solid Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Herpesviruses: To determine a possible co-carcinogenic role for herpesviruses in transformation induced by chemical carcinogens.

Previous Serial Number: None

Principal Investigator: Dr. Ashley Brown

Other Investigators: Dr. Berge Hampar

Cooperating Units: Inside NIH

None

Outside NIH

None

Man Years:

Total:	0.9
Professional:	0.9
Other:	0.0

Project Description

Objectives:

(1) To determine a possible co-carcinogenic role for herpes simplex virus in chemically induced transformation.

Major Findings:

This project has only recently been initiated and most of the effort to date has involved development of the systems.

(1) Cell transformation by chemical carcinogens with or without added HSV: Evidence exists that HSV may enhance the rate of tumorigenesis induced by chemical carcinogens in animals. Efforts have been made to develop an in vitro system for determining a possible co-carcinogenic role for HSV in chemically induced transformation using BALB/3T3 cells and cells isolated from rat submaxillary gland (RSM).

The drugs employed for transformation include polycyclic hydrocarbons and dimethylnitrosoamine, and a temperature sensitive (Ts) mutant of HSV has been prepared for assessing the role of virus infection on cell transformation. Preliminary experiments have been carried out to determine optimal conditions for obtaining and isolating cell transformants. Once these conditions have been established more definitive experiments will be carried out.

Significance to Biomedical Research and the Program of the Institute:

A possible oncogenic role for herpesvirus in human tumors needs to be clarified. This would include either a direct oncogenic role of the herpesvirus genome or an indirect co-carcinogenic role.

Proposed Course:

To continue development of adequate in vitro systems and to initiate definitive studies on a possible co-carcinogenic role of HSV.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-0071D-12-VC
1. Viral Diseases
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Mouse Leukemia Viruses

Previous Serial Number: Same

Principal Investigators: Dr. Janet W. Hartley, LVD, NIAID
Dr. Wallace P. Rowe, LVD, NIAID

Other Investigators: Dr. Sisir K. Chattopadhyay, LVD, NIAID
Dr. Stephen Staal, LVD, NIAID
Dr. Akinori Ishimoto, LVD, NIAID (from Sept. 1974)
Dr. Ernest Manders, LVD, NIAID
Dr. Rex Risser, LVD, NIAID (from Feb. 1975)

Cooperating Units: Inside NIH:

Dr. Tadao Aoki, VLL, NCI
Dr. Ronald Herberman, LCBGY, NCI
Dr. Arthur S. Levine, DCT, NCI
Dr. Wade Parks, VC, NCI
Dr. Michael Potter, LCBGY, NCI
Dr. Edward Scolnick, VLL, NCI

Outside NIH:

Dr. Edward A. Boyse, Sloan Kettering Institute for Cancer Research, New York, New York
Dr. Theodore Bremner, Howard University, Washington, D.C.
Dr. H. Ikeda, Sloan Kettering Institute for Cancer Research, New York, New York
Dr. Frank Lilly, Albert Einstein College of Medicine, New York, New York
Dr. Douglas R. Lowy, Yale University School of Medicine, New Haven, Connecticut
Dr. Michael N. Oxman, Children's Hospital Medical Center, Boston, Massachusetts
Dr. John C. Parker, Microbiological Associates, Inc., Bethesda, Maryland
Dr. Paula Pitha, Johns Hopkins University, Baltimore, Maryland
Dr. Marshall D. Sklar, Stanford University Hospital, Stanford, California
Dr. E. Stockert, Sloan Kettering Institute for Cancer Research, New York, New York

Man Years:

Total:	12.75
Professional:	4.0
Other:	8.75

Project Description

Objectives:

To utilize sensitive procedures for genome detection and isolation and quantitation of infectious virus in studying the natural history of murine leukemia virus (MuLV) infection.

Methods Employed:

Tissue culture procedures, using plaque and transformation assays; tests for antigen induction by complement fixation and fluorescent antibody procedures; reverse transcriptase assays. Mouse inbreeding, to select for specific virus-inducing or virus-related genes. Starch-gel electrophoresis for assays for marker isoenzyme loci. Nucleic acid hybridization techniques.

Major Findings:

Biochemical studies of MuLV infection: In studies of the viral nucleic acid sequences of mouse-tropic AKR-type murine leukemia virus detectable in high-, low-, and non-virus-yielding mouse strains, it was found that the complete AKR virus genome is present in the DNA of high-virus-yielding strains (AKR, C_3H/Fg) and low-virus-yielding strains (BALB/c, DBA/2, C_3H/He, and $C_{57}Bl/6$), while the DNA of non-virus-yielding mice (NIH Swiss, 129/J, $C_{57}L/J$ and NZB/N)contains only a part of the genome. In strains with the complete genome, the reassociation rates of the DNA's with the single-stranded AKR viral DNA probe (complementary to AKR viral RNA) indicate two populations of virus-specific DNA sequences, one more abundant and one less abundant. The DNA from low-virus-yielding strains contains fewer copies of the less abundant species while that from non-virus-yielding strains lacks a major portion of the viral genome, apparently corresponding to the less abundant set of sequences.

Proof that one mouse-tropic virus inducing locus in AKR mice, Akv-1, contains viral genetic material was obtained by demonstrating that AKR-type viral DNA sequences correlated completely with presence of Akv-1 in mice segregating for this locus.

Studies of the $Fv\text{-}1^n$ locus: In the original studies describing the genetic basis of resistance of cells from various mouse strains to exogenous murine leukemia virus, it was noted that among $Fv\text{-}1^n$ mouse strains (i.e., strains sensitive to N-tropic virus but markedly resistant to B-tropic virus) some showed decreased sensitivity to N-tropic virus. It now appears that this

property is controlled by an allele of the Fv-1^n gene locus, is found in the 129, RF, NZB, and NZW strains, and is apparently segregating in NIH Swiss, General Purpose Swiss, and the HSFS/N inbred NIH Swiss line. This poorly permissive FV-1^n allele is able to restrict growth of genetically transmitted N-tropic virus in hybrid mice almost as effectively as Fv-1^b, and consequently is a major variable in infectious expression of Gross-AKR virus.

Genetics of N- and B-tropic virus induction in B10.Br mice: The congenic mouse strain B10.Br/Li is a B-type mouse which has a high frequency of predominantly B-tropic virus in vivo; IdU induction of tissue culture cells results in a high frequency of N-tropic virus and occasionally B-tropic virus production. Preliminary studies of the backcross progeny of matings of B10.Br and A strain (a B-type, low-virus-yielding strain) indicate that B10.Br possesses two independent loci for induction of N-tropic virus, one of which is also associated with B-tropic virus induction.

Detection of a new class of murine leukemia viruses: When it was found that MuLV isolates from Los Angeles area feral mice were capable of infecting heterologous species cells, limiting dilution purification studies were carried out in attempts to resolve possible mixtures. It has been found that several isolates of feral MuLV contain two components, an N-tropic virus whose host range is restricted to mouse and rat cells, and a virus population, termed "amphitropic", which can propagate in N-type mouse cells and in a wide variety of heterologous species cells. The heterologous cell host range of amphitropic virus is similar to that of xenotropic viruses except that it does not infect duck or quail cells. Using MSV pseudotypes of mouse-tropic, xenotropic, and amphitropic MuLV, it has been found that the three host-range classes can be distinguished on the basis of interference activity, i.e., that representatives of each class do not significantly interfere with each other.

Assay systems for xenotropic viruses: The commonly used assays for xenotropic virus have been by MSV rescue or determination of reverse transcriptase activity; neither method is satisfactory for direct quantitation of xenotropic MuLV yields. We have found that quantitation is possible by enumeration of foci of fluorescent cells in cultures infected with dilutions of xenotropic virus; acetone-fixed preparations are stained with a direct test with fluorescein-conjugated anti-Moloney serum (obtained from Dr. Roger Wilsnack, Huntingdon Research Center). Using this test, several cell systems, including human, rabbit (SIRC), and mink have been compared for sensitivity to a number of xenotropic MuLV. The results of FA focus assays in SIRC cells correlate well with assays in the feline S+L-line recently described by others, while FA assays in mink lung cells have been consistently superior in sensitivity, particularly for the virus released by NZB cell lines which is poorly infectious for other cell lines tested.

Significance to Biomedical Research and the Program of the Institute:

The development of methods for virus detection, propagation, and quantitation is essential to studying the natural occurrence and role in tumorigenesis of the murine leukemia viruses. The quite recent recognition of the xenotropic and amphitropic viruses is an example of the continuing revelation of the complexities of murine leukemia virus infection and the incompleteness of present knowledge.

The application of precise genetic and biochemical methods to the study of integration of viral genetic information may permit the correlation of specific virus sequences with development of particular types of tumors. Comparative study of the genetic information specified by different classes of murine leukemia viruses in various strains of mice is now feasible and it may be possible to determine the evolutionary origin of MuLV infection.

Proposed Course:

Studies will continue on localizing the viral integration sites in various strains of mice and on the genetic relationship of various markers of MuLV expression and control.

Biochemical studies will attempt to distinguish between viral sequences specified by different murine leukemia viruses in the DNA of mice with differing expression of MuLV information. DNA from other rodent species will be tested for homology with MuLV viral DNA probes.

We will attempt to classify mouse-tropic AKR-Gross, FMR, and feral isolates, xenotropic viruses, and amphitropic feral isolates by neutralization testing and, in collaboration with Dr. Wade Parks, by analysis of viral proteins. The feral mouse-tropic and amphitropic isolates will be tested for ability to induce tumors and/or neurologic disease, in mice, in attempts to reproduce the unusual paralytic disease induced by certain feral MuLV isolates, as described by Gardner and Huebner.

The interaction of amphitropic viruses with mouse-tropic viruses will be studied in attempts to detect genetic recombination.

Studies of the ability of Abelson virus and other MuLV to transform lymphocytes and other cell types will be resumed.

Publications:

Chattopadhyay, S.K., Lowy, D.R., Teich, N.M., Levine, A.S., and Rowe, W.P.: Qualitative and Quantitative Studies of AKR-Type Murine-Leukemia-Virus (MLV) Sequences in DNA of High-, Low-, and Non-Virus-Yielding Mouse Strains. Cold Spring Harbor Symposium Quant. Biol., Vol. 39, 1974. In Press.

Chattopadhyay, S.K., Rowe, W.P., Teich, N.M., Lowy, D.R.: Definitive evidence that the murine C-type virus inducing locus Akv-1 is viral material. Proc. Natl. Acad. Sci., 1974. In press.

Hartley, J.W. and Rowe, W.P.: Clonal Cell Lines from a Feral Mouse Embryo which Lack Host-Range Restrictions for Murine Leukemia Viruses. Virology, 1974. In press.

Lilly, F., Duran-Reynals, M.L., and Rowe, W.P.: Correlation of Early Murine Leukemia Virus Titer and H-2 Type with Spontaneous Leukemia in Mice of the BALB/c x AKR. J. Exp. Med., 1974. In press.

Lowy, D.R., Chattopadhyay, S.K., Teich, N.M., Rowe, W.P., and Levine, A.S.: AKR murine leukemia virus genome: Frequency of sequences in DNA of high-, low-, and non-virus-yielding mouse strains. Proc. Natl. Sci., 71: 3555-3559, 1974.

Nomura, S., Dunn, K.J., Mattern, C.F.T., Hartley, J.W., and Fischinger, P.J.: Revertants of mouse cells transformed by murine sarcoma virus from a clone of BALB/3T3 transformed by Kirsten MSV. J. of General Virology, 25: 207-218, 1974.

Pitha, P.M., Pitha, J., and Rowe, W.P.: Lack of requirement of reverse transcriptase function for the activation of murine leukemia virus. Virology, 1974. In press.

Sklar, M.D., White, B.J., and Rowe, W.P.: Initiation of oncogenic transformation of mouse lymphocytes in vitro by Abelson leukemia virus. Proc. Natl. Acad. Sci. 71: 4077-4081, 1974.

SUMMARY REPORT

c. VIRAL LEUKEMIA AND LYMPHOMA BRANCH
July 1, 1974 - June 30, 1975

The Viral Leukemia and Lymphoma Branch conducts research designed to elucidate the role of viruses in the etiology of human neoplasms, particularly leukemias, lymphomas and sarcomas. A variety of scientific approaches are used which provide a broad base of knowledge applicable to the identification and isolation of human oncogenic agents and the prevention or control of the disease as it occurs in man. More specifically, the Branch encompasses a range of scientific disciplines including molecular biology, genetics, immunology, biochemistry, pathology, and cell culture techniques. In the past year, the emphasis has been away from model systems to the more direct study of human materials.

The Viral Pathology Section studies in vivo and in vitro aspects of viral oncogenesis, including virology, immunology, pathogenesis and the interferon system, emphasizing cellular control mechanisms of viral replication. The Genetics Section seeks to obtain comprehensive knowledge of the genetics and biochemistry of sarcoma and leukemia viruses and conducts quantitative studies on the interaction of oncogenic viruses and cells to determine the mechanisms of viral replication and cellular transformation at the molecular level. The Primate Virus Section conducts studies to determine the pathologic factors of both tumor viruses and the hosts which they infect, that are involved in the oncogenic process. Particular emphasis is placed on herpes virus oncogenesis in primates. The Viral Biochemistry Section plans and conducts studies to detect viruses and their constituents in human tumor cells and model systems by biological and biochemical methods. The Immunology Section examines the antigenic nature of oncogenic viruses and their induced tumors as well as the immune response of the host to viral infection and tumor development. The Tumor Virus Section is concerned with defining in detail the biological and biochemical properties of tumor viruses in order to understand how they may be applied to the search for human tumor viruses. A helper assay to rescue oncogenic virus information is currently being applied to human cell systems. The Clinical Studies Section utilizes information and laboratory techniques derived from investigations in viral oncology and applies these techniques to clinical studies on the etiology and control of cancer. The Office of the Chief coordinates the research of the various sections while recognizing the scientific freedom of the individual investigators. The Office establishes collaborative efforts with investigators in other areas of NIH and elsewhere such that information derived from studies within the Branch is constantly being applied in investigations leading to a better understanding of the etiology of human neoplasia.

Endogenous type C viruses have been isolated from three different baboon species (genus Papio) by cocultivation of various tissues and cell lines with suitable heterologous mammalian cells. These viruses are distinctly different by immunologic and nucleic acid hybridization techniques from previously studied mammalian type C viruses. With ^{3}H-DNA transcripts prepared from the endogenous baboon type C viruses, partially homologous sequences have been detected in the cellular DNA of all species of the primate suborder Anthropoidea (Old World monkeys, apes, and man). This demonstrates that all Old World monkey and great ape families (including the human family Hominidae) possess endogenous type C virogenes, and, thus, the potential for producing endogenous type C viruses. Within the primates, type C viral genes have evolved as the species have evolved, with virogenes from more closely related genera and families having more sequence homology than those from distantly related taxons. Type C viral probes offer distinct advantages as tools for the study of vertebrate evolutionary relationships.

Antigens related to the major structural protein (p30) of baboon type C viruses have also been detected in normal baboon, rhesus monkey, and stumptail monkey tissues, and more recently, in several human tumors. These results indicate that humans, like other primates, contain endogenous type C virogenes in their DNA which can, in certain circumstances, be at least partially expressed.

A reverse transcriptase has been purified from three cases of human acute myelogenous leukemia in collaboration with Dr. Robert Gallo's group. The enzymes have antigenic properties closely related to those of the type C viruses found in woolly monkeys and gibbon apes. The gibbon ape and woolly monkey viruses, which are highly infectious for primates, do not seem to be endogenous primate viruses and may have at one time been derived from rodents. However, the enzyme in the particles in the human leukemic cells has the properties of a type C viral enzyme, and its reverse transcriptase is particularly strongly inhibited by antisera to the gibbon ape type C reverse transcriptase group.

By several criteria, including nucleic acid sequence homology and antigenicity of the polymerase and the p30 protein, the various baboon viruses are related to, but distinct from, the endogenous cat viruses of the RD-114/CCC group. The unexpected relatedness of the endogenous cat and the endogenous baboon viruses suggested that they had a common origin. All members of the cat family Felidae (36 species belonging to six genera) are closely related as shown by hybridization of their cellular DNA. Only four species of the genus Felis (domestic cat, sand cat, jungle cat, and European wildcat) contain endogenous virogenes related to the RNA of RD-114. However, all Old World monkey and ape species examined have nucleic acid sequences related to RD-114. These findings suggest that viral genes from primates gave rise to infectious particles that not only integrated into the DNA of an ancestor of the domestic cat but were incorporated into the germ line and transmitted as cellular genes. By examining the divergence time of the various species discussed above, this infection is believed to have occurred about 5-7 million years ago in the Mediterranean basin.

It is characteristic of endogenous type C viruses that all individual animals of a species, as well as all tissues of each animal, contain, in the chromosomal DNA, nucleic acid sequences that can code for the production of complete type C viral particles. By molecular hybridization, the reiteration frequency was examined of mouse, rat, pig, cat, and baboon viral nucleic acid sequences in tissues and cell cultures of the species of origin of these viruses and in exogenously infected cells of heterologous species. While DNA from each species contains multiple (5-15) copies of endogenous virogene sequences per haploid genome, cell lines of heterologous species infected with and producing high titers of these type C viruses contain fewer copies (one or two per haploid genome). The thermal stability of DNA:DNA hybrids demonstrates that the endogenous virogenes are composed of families of genes with related but not identical nucleic acid sequences. The presence of multiple copies of viral information is thus a useful criterion with which to identify the species of origin of an endogenous mammalian type C virus.

N-tropic and xenotropic type C viruses have been isolated from the wild Asian mouse subspecies M. musculus molossinus. By host range, morphologic and some immunologic criteria these viruses appear closely related to the previously studied murine type C viruses isolated from highly inbred laboratory strains of mice.

The nucleic acid sequence homology between various murine, endogenous type C viruses and two laboratory strains of murine leukemia virus was determined by DNA:RNA hybridization. The viral sequences exhibit varying degrees of partial homology. Cellular DNAs from BALB/c, NIH Swiss, and AKR inbred mouse strains as well as from California feral mice and the Asian mouse subspecies Mus musculus molossinus and M. musculus castaneus, all possess multiple (six to seven per haploid genome), partially related copies of type C virogenes in their DNAs. Thermal melting profiles of the DNA:RNA and DNA:DNA hybrids suggest that the partial homology of the viral nucleic acid sequences is the result of base alterations throughout the viral genomes, rather than the loss of discrete segments of viral sequences.

Nonproducer cells have been isolated containing the sarcoma viral genome of the Moloney sarcoma virus, the Kirsten and Harvey strains of sarcoma virus, several strains of feline sarcoma virus, and woolly monkey sarcoma virus. Mammalian RNA sarcoma viruses are type C viruses because they contain as part of their genome, nucleic acid which is homologous to replicating type C viruses. This is true in each case of the above named sarcoma viruses. Interestingly, the woolly monkey sarcoma virus contains information homologous to the woolly leukemia virus and this information is more closely related to the woolly leukemia virus than to any of the known gibbon ape leukemia viruses. The feline sarcoma viruses contain feline leukemia information but no information homologous to another feline virus RD-114. The murine sarcoma viruses contain nucleic acid information homologous to the original leukemia virus present in the stock used to isolate the virus.

The Kirsten and Harvey strain of sarcoma virus (which were each isolated by passage of MuLV in rats) contain genetic information not present in either

the Kirsten strain of leukemia virus or the Moloney strain of leukemia virus. The additional information present in the Kirsten and Harvey viruses comes from rat cells and is present in normal rat cellular DNA. Apparently, the rat genetic information present in the Kirsten and Harvey viruses is the same. Thus, in two independent isolates of transforming viruses, a gain of rat genetic information has occurred. The rat genetic information in these two sarcoma viruses is distinct from the known rat type C viral nucleic acid sequences and is present in normal rat cells. The question is unresolved as to whether the new rat genetic information is of cellular origin or represents a distinct type C virus in rats different from the known rat type C viruses.

In collaboration with Dr. Peter Duesberg of the University of California in Berkeley it has been shown that the mouse and rat information contained in the Harvey sarcoma virus are covalently linked. These experiments directly prove in a way that has not been possible up to now that RNA tumor viruses are capable of undergoing the classic type of genetic recombination by crossing-over which leads to covalent linkage of distinct genetic information.

True revertants have been obtained from sarcoma transformed (S+) cat cells. These had properties of normal parental CCC cells, showed a variable expression of the endogenous CCC genome, and have lost the p30 MuLV marker carried by the sarcoma genome. Human S+L- cells also gave rise to flat variants, but even after multiple cycles of cloning all flat variants proved to be rescuable. Thus only phenotypic reversion was found in human cells.

The most efficient method of elicitation of endogenous mouse xenotropic virus was the coinfection with some strains of MuLV. Thus even outbred mouse cells previously not inducible by chemical means now released xenotropic type virus.

The FeLV pseudotype of MSV contained a highly atypical p60 polypeptide. This protein, not found in MSV(MuLV), contained at least MuLV p30 group and interspecies determinants. It could be cleaved enzymatically to lower molecular weight polypeptides which also retained MuLV p30 reactivity. Apparently a cleavage defect exists in the cat cell for MSV.

The presence of sarcoma cell surface antigen (SCSA) on three different cell lines (Kirsten sarcoma virus-transformed nonproductive BALB/3T3 (K-BALB)), and (normal rat embryonic kidney (NRK) transformed by Kirsten and woolly monkey sarcoma virus to nonproducer sarcoma cells (K-NRK and W-NRK)) has been examined by immunoelectron microscopy using rabbit anti-K-BALB, anti-K-NRK, and anti-W-NRK sera preabsorbed with normal embryonic rat cells, NRK cells, and BALB/3T3 cells. Four different specific SCSAs have been demonstrated: SCSAa, an antigen specific to the surface of W-NRK cells; SCSAb, a surface antigen common to K-NRK and W-NRK cells; SCSAc, a surface antigen common to K-BALB and W-NRK cells; and SCSAd, a surface antigen broadly reactive with the rabbit antisera to W-NRK, K-NRK, and K-BALB.

Peripheral blood platelets of quail and mice, when injected together with sarcoma viruses enhanced the growth of Rous sarcomas in chicken and Moloney sarcomas in BALB/c mice respectively. The effective factor appears to be contained in the supernatant of platelet homogenate after low-speed centrifugation.

Selected sera of normal gibbon apes reacted with the entire envelope of all type C viruses released from a cultured gibbon leukemia cell line and with small areas of the cell surface. After adaptation of these viruses to a human rhabdomyosarcoma cell culture, one virus population reacted positively with the gibbon sera but another virus population did not react.

MSV pseudotypes produced after MuLV superinfection of nonproducer or S+L- cells display N-, B-, or NB-tropism similar to murine leukemia virus isolates. In all cases studied (Moloney S+L-, Moloney nonproducer, Kirsten, and Harvey) the tropisms of MSV pseudotypes were identical to those of the MuLV strains used for rescue. 3T3FL cells, which are dually susceptible to N- and B-tropic MuLV's are also equally susceptible to the corresponding MSV pseudotypes . As is the case with resistance to tropic MuLV, resistance to MSV pseudotypes is not absolute, since foci are always found in nonproducer cells. A factor(s) which is responsible for MuLV susceptibility or resistance to Fv-1 restriction is apparently transferred to the MSV virion during the rescue process. This factor is not a stable part of the MSV genetic information and may be analogous to other phenotypically acquired properties, such as neutralizing antigens and host range. MSV "pseudotypes" are tropic when quantitatively assayed by techniques which measure either virus replication or cell transformation.

Cells which are producing RD-114 virus contain two forms of viral reverse transcriptase. One, the normal form, is packaged in the virion and is 70,000 daltons, and the second, of about 90,000 daltons, which is a minor species in the infected cell and is apparently not packaged in the virion. The polymerases are indistinguishable antigenically.

Further studies on American Burkitt lymphoma patients identified by the American Burkitt Lymphoma Registry revealed similarities between American and African cases. About 80% of patients with Stage I disease were demonstrated to have long-term survival and no longer required chemotherapy. An excellent response rate was also noted for Stage II patients. The overall long-term remission rate was 33%, comparable to that found in Ghana. An EBV producing cell line was obtained from an American BL patient with significant amounts of detectable EBV genome in her tumor.

Twenty-four nasopharyngyal cancer patients from France and Tunisia were skin tested with membrane extracts from cell lines derived from NPC and BL tumors. More that 50% of patients reacted against HKLY-28, a cell line derived from an NPC patient, but a much lower percentage reacted against Raji (a BL-derived line) and cell lines derived from normal individuals, indicating a tumor associated antigen was present in the NPC line.

Twenty-five sera from 21 patients with American Burkitt lymphoma were assayed for antibodies to four different EBV antigens: VCA (viral capsid antigen), CF (particulate complement fixing antigen), EA (early antigen) (15 sera only), and NA (nuclear antigen). The American Burkitt sera had higher geometric mean titers (GMT) than the controls to all four antigens. In the case of NA, the GMT of the American Burkitt sera was 59, while that of the ten controls was 1.5. Although 11 of 25 of the American Burkitt sera had negative titers for one or more antibodies, none of 15 sera were negative for all four antibodies.

Two soluble (S) complement fixing (CF) EBV antigen complexes were isolated from ^{14}C-labelled cell cultures of P_3HR-1 (EBV producer). The first antigen complex is further separable into two components with molecular weights of 40,000 and 200,000 daltons. The second antigen component is a single protein with a molecular weight of 70,000 daltons. The crude S antigen is stable at -70°C at least for three months and is partially resistant to heat (56°C for one hour). The CF activity is readily destroyed by treatment with trypsin, acetone, chloroform, and pronase. Lipase and phospholipase did not completely destroy the CF activity of the S antigen but reduced it significantly.

Xenotropic strains of MuLV have been subtyped on the basis of host range. Viruses from BALB/c appear to have a relatively restricted host range and replicate most efficiently in SIRC cells ("S tropic" viruses). Those from NIH Swiss, and certain other strains, have an extensive host range and replicate efficiently on human as well as SIRC cells. Neither type grows in mouse, wild mouse or human x mouse hybrid cells. Another subtype of xenotropic virus has been isolated from certain FMR virus stocks. This subtype has an extensive host range, but also replicates in wild mouse (SC-1) and human x mouse hybrid cells. All the xenotropic subtypes are negative in the XC and S+L- assays, and they cannot be distinguished by interference or neutralization tests. These data and those obtained from MSV pseudotypes indicate that xenotropic virus replication control is more complex than N, B tropism. Virus penetration can only be demonstrated in human-mouse hybrids and SC-1 cells but not in N or B cells. Intracellular restriction is present in all mouse cells and hybrid cells. The subtypes isolated from FMR virus stocks are subject to different control mechanisms. Their envelope (and host range) are similar to other xenotropes, however, they are not subject to intracellular control. Hence they can replicate in SC-1 cells and in human x mouse hybrid cells. Previous work demonstrated an intracellular block to replication of the xenotropic strain AT-124. When human cells preinfected with AT-124 are fused with N and B cells the hybrid clones generated continue to release virus. These experiments strongly suggest that the block occurs at a "preintegration" level. In summary, it appears that naturally occurring xenotropic MuLV strains are subject to two control mechanisms - at the penetration level as well as at an intracellular preintegration level. While neither mechanism may be absolute, the net result is an absolute block.

In addition to their intramural research activities, several of the senior investigators within the Branch spent a substantial portion of their time in support of the Virus Cancer Program. The members serve as Chairmen, Vice-Chairmen, and Executive Secretaries of Segments of the Program. They provide scientific guidance as Project and Assistant Project Officers on research contracts supported by the Virus Cancer Program. The activities of the VCP and the direction of the internal research program of the Branch are aimed at the common goal of the determination of the viral etiology of human cancer. It is apparent that the efforts of the Branch members have played a significant role in the progress of the VCP to date. The broad scientific perspective developed by these investigators in their VCP activities has also contributed significantly to the direction of the Branch program for the attainment of research goals. The effective functioning of senior personnel in dual capacities, i.e., in-house research and program

administration, requires a delicate balance of effort. It must be realized through constant monitoring, that such a balance does exist and over-emphasis in either direction would be to the detriment of both programs. It has become clearer during the past year that an understanding of the suspected relationship between tumor viruses and human neoplasia requires an interaction between, among others, highly skilled molecular biologists, virologists, epidemiologists, cell biologists, and physicians, along with sound and constructive administrative support; the answers will come from no one discipline alone.

Serial No. Z01-CP-04821-03-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Genetic and environmental factors in susceptibility to endogenous and exogenous tumor virus information

Previous Serial Number: Same

Principal Investigator: Dr. George J. Todaro

Other Investigators: Dr. Raoul Benveniste
Dr. Robert Callahan
Dr. Joseph DeLarco
Dr. Charles J. Sherr

Cooperating Units: Inside NIH
Dr. Robert Gallo, LTCB, NCI
Dr. Roscoe Brady, DMN, NINDS
Dr. Michael Potter, LCB, NCI
Dr. Douglas Key, D, NCI
Dr. Robert Huebner, VC, NCI
Dr. Wade Parks, VC, NCI
Staff, VLL, NCI

Outside NIH
Meloy Laboratories, Springfield, Virginia
Electro-Nucleonics, Bethesda, Maryland
Naval Biological Research Laboratory, Oakland, California
Baylor College of Medicine, Houston, Texas
Animal Diseases Research Institute, Ottawa, Canada

Man Years:

Total:	3.00
Professional:	1.00
Other:	2.00

Project Description

Objectives:

1. Isolate and characterize endogenously and exogenously transmitted type C viruses from man, primates and other animals.

2. Determine the mechanism by which cells prevent and/or express endogenous oncogenic virus information.

3. Study how agents such as radiation, chemical carcinogens and viruses act alone or in concert to facilitate cell transformation.

4. Study the ability of RNA and DNA from tumor viruses to transform human cells.

5. Determine the basis for the difference in susceptibility of cells to exogenously added oncogenic RNA viruses.

6. Characterize endogenous and horizontally transmitted type C viruses and their relationship to "spontaneous transformation" in cell culture and in the animal.

Methods Employed:

Standard techniques of tissue culture, biochemistry, and immunology, virus isolation and virus antigen detection are being used.

Major Findings:

1. Type C viruses have been isolated from baboon tissues. The first isolate was obtained from a baboon placenta and the virus was found to grow well in a variety of heterologous cells, but especially in a dog thymus line (FCf2Th). Several additional viruses were isolated from baboon spleen, lung, and testes. The DNA product of an endogenous reaction hybridizes to normal baboon cell DNA and also to the cellular DNA of other primates including man. Viral expression (the p30 protein) can be detected in normal baboon spleen, testes, and placenta and in other primates, including man. Eight to 12 DNA copies are found per diploid genome in all normal baboons tested. This is the first demonstration of a group of endogenous type C viruses in primates. The finding of related genetic information in other primates indicates that they, too, have endogenous type C virus information. Humans, like other primates, have type C viral sequences in their genome and can, in some circumstances, express at least one type C viral protein.

2. A reverse transcriptase has been purified from three cases of human acute myelogenous leukemia in collaboration with Dr. Robert Gallo's group. The enzymes have antigenic properties closely related to those of the type C viruses found in woolly monkeys and gibbon apes. The gibbon ape and woolly monkey viruses, which are highly infectious for primates, do not seem to be endogenous primate viruses and may have at one time been derived from rodents. However, the enzyme in the particles in the human leukemic cells has the properties of a type C viral enzyme, and its reverse transcriptase is particularly strongly inhibited by antisera to the gibbon ape type C reverse

transcriptase group. If further cases of leukemia show the same characteristic enzyme, it would strongly implicate infectious, as opposed to endogenous, type C viruses in human acute leukemia.

3. Type C viral probes offer distinct advantages as tools for the study of vertebrate evolutionary relationships. Cellular DNAs from all species of the primate suborder Anthropoidea studied contain base sequences partially homologous to genes of endogenous baboon type C virus. This demonstrates that all Old World monkey and great ape families (including the human family Hominidae) possess endogenous type C virogenes and, thus, the potential for producing endogenous type C viruses. Within the primates, type C viral genes have evolved as the species have evolved, with virogenes from more closely related genera and families showing more sequence homology than those from distantly related taxons. The apparent rapid evolution of type C virogenes permits a quantification of evolutionary divergence among the genera of the Old World monkey subfamily Cercopithecinae despite the closeness of these genera, as measured by overall unique sequence DNA.

4. There is partial sequence homology between the cat (RD114/CCC) virus group and the baboon virus group; the gs antigens and reverse transcriptases of viruses of these two groups share antigenic determinants with one another and their pseudotypes interfere with one another. The results indicate a closer evolutionary relationship between these two groups of viruses that would not have been expected based on the extensive genetic divergence between the species. Since genes related to RD114 are found in the cellular DNA of anthropoid primates while many members of the cat family Felidae lack these sequences, endogenous viruses from primates are thus concluded to have infected and become part of the germ line of an evolutionarily distant group, the ancestors of the domestic cat.

5. The reiteration frequency of mouse, rat, pig, cat and baboon viral nucleic acid sequences have been examined in tissues and cell cultures of the species of origin of these viruses, and in exogenously infected cells of heterologous species. While DNA from each species contains multiple copies of endogenous virogene sequences per haploid cellular genome (5-15), cell lines of heterologous species infected with and producing high titers of these type C viruses contain fewer copies (1-2 per haploid genome). The thermal stability of DNA:DNA hybrids demonstrates that the endogenous virogenes are composed of families of genes with related but not identical nucleic acid sequences.

6. N-tropic and xenotropic type C viruses have been isolated from the wild Asian mouse subspecies M. musculus molossinus. By host range, morphologic and some immunologic criteria these viruses appear closely related to the previously studied murine type C viruses isolated from highly inbred laboratory strains of mice.

7. The nucleic acid sequence homology between various murine, endogenous type C viruses and two laboratory strains of murine leukemia virus was determined by DNA:RNA hybridization. The viral sequences exhibit varying

degrees of partial homology. Cellular DNAs from BALB/c, NIH Swiss, and AKR inbred mouse strains as well as from California feral mice and the Asian mouse subspecies Mus musculus molossinus and M. musculus castaneus, all possess multiple (six to seven per haploid genome), partially related copies of type C virogenes in their DNAs. Thermal melting profiles of the DNA:RNA and DNA:DNA hybrids suggest that the partial homology of the viral nucleic acid sequences is the result of base alterations throughout the viral genomes, rather than the loss of discrete segments of viral sequences.

8. An endogenous type C virus has been isolated from a pig cell line (PK15). The reverse transcriptase and the major gs antigen have been purified and specific reagents have been prepared to it. Using these, it is possible to detect expression of type C virus information in pig tissues. This virus can be readily distinguished from other mammalian type C viruses, and DNA copies of the viral information are found in normal pig tissue. Late passages of the PFT porcine oviduct cell line spontaneously release a typical type C virus antigenically related to the type C virus released from the PK(15) porcine kidney cell line. The PFT virus appears to have poor or absent infectivity. Type C virus can also be induced by BrdU treatment from earlier passages of the PFT cell line.

9. Fifteen of 18 cell lines established from normal mouse cells and transplantable tumors produced infectious type C viruses. For all cell lines examined, the viral isolates were either N-, B-, or NB-tropic, while xenotropic viruses were not detected. Two cell lines derived from normal tissues, however, had xenotropic viruses which could be induced by treatment with 5-bromodeoxyuridine, whereas cell lines derived from transplantable tumors were non-inducible. Nine standard leukemia virus preparations likewise did not contain xenotropic viruses. In contrast, xenotropic viruses were recovered from spleens of normal weanling mice from six of 13 strains, indicating that the expression of xenotropic virus is relatively common in disease-free animals.

10. Because of its flat regular growth in monolayer culture and its broad host range as a permissive cell for replication of a variety of mammalian type C viruses, the MvlLu mink cell line (CCL 64) has been found to be useful for focus forming assays of murine and feline sarcoma viruses, the generation of nonproducer murine and feline sarcoma virus-transformed cell lines, and the construction of sarcoma virus pseudotypes of mammalian type C viruses.

11. Chemically and X-ray transformed subcones of Balb/c 3T3 mouse embryo cells were found to have reduced amounts of the mono-and disialo-gangliosides galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide (G_{M1}) and N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide (G_{D1a}) and increased amounts of N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide (G_{M2}). The activity of the enzyme UDP-Gal:G_{M2} galactosyltransferase was reduced to between 2.7 and 14.3% of normal in the transformed clones. Other ganglioside glycosyltransferase activities were unaffected. This enzymatic change was consistent with the observed alteration in ganglioside pattern in the

transformed cells. The residual galactosyltransferase activity in the transformed cells was kinetically similar to the normal enzyme, suggesting that transformation alters ganglioside biosynthesis by blocking enzyme synthesis at the translational or transcriptional levels.

12. Both human xeroderma pigmentosum skin fibroblasts and normal skin fibroblasts were exposed to low-dosage UV radiation immediately prior to infection with SV40; the incidence of T-antigen, an early marker of SV40 infection, and of gross transformation, the morphologic late end point in vitro, were noted. SV40 T-antigen induction was not increased post irradiation. No increase in transformation either absolute or relative to cell survival post irradiation was seen.

Significance to Biomedical Research and the Program of the Institute:

These projects relate to the nature of the interaction between tumor viruses and the cells that contain them. Development of techniques in the model systems are frequently applicable to the search for human oncogenic viruses. The methods by which the Program can hope to detect or to prevent virus-induced tumors will depend on how the viruses produce their effect. Two very different models must be considered; they act via the addition of genetic information to the cell from the outside, or, via activation of previously repressed genetic information. Which of these is the more critical under natural conditions for cancer in man is of paramount importance in determining how best to interrupt or prevent this process.

Proposed Course:

1. Specific reagents will be prepared to each of the major components of the primate type C viruses to use to study the evolutionary relationship among the primates and the extent of expression of virus specific information in normal humans and in humans with tumors, expecially leukemias and lymphomas.

2. To define the level at which cells prevent the expression of endogenous virus information and to determine where in the cell the virus information physically resides. To develop techniques that utilize radiation, chemical carcinogens, and viruses to produce human cell transformation and/or activation of type C virus in tissue culture. In addition, the project will be directed towards finding specific repressor substances that may control type C viral expression.

3. A combination of methods that have recently become available will be used to try to resolve whether type C viruses are present both in human tumor cells and in normal human cells. If they are, attempts will be made to determine how they are transmitted and their importance as etiologic agents in human cancer. The factors responsible for both susceptibility and resistance to viral infection will be studied with the goal of characterizing these factors in biochemical terms. Simple in vitro tests measuring a person's risk of developing cancer in reasonably large population samples will hopefully resolve

whether major differences exist between normals and those that actually develop cancer.

4. To characterize the biology and immunology of the endogenous and infectious type C viruses of each of the mammalian species from which viruses have been or will be isolated. In order to determine their relationship to one another and to normal cellular genes.

Honors and Awards:

Editorial Board member of the scientific journals entitled, Intervirology, Cell, and Cancer Research.

Publications:

Key, D.J. and Todaro, G.J.: Xeroderma pigmentosum susceptibility to SV40 virus transformation: Lack of effect of low dosage ultraviolet radiation in enhancing viral induced transformation. J. Invest. Dermatol. 62: 7-10, 1974.

Todaro, G.J., Benveniste, R.E., Lieber, M.M., and Sherr, C.J.: Characterization of a type C virus released from the procine cell line PK(15). Virology 58: 65-74, 1974.

Gallagher, R.E., Todaro, G.J., Smith, R.G., Livingston, D.M., and Gallo, R.C.: Relationship between reverse transcriptase from human acute leukemic blood cells and primate type C viruses. Proc. Natl. Acad. Sci. USA 71: 1309-1313, 1974.

Lieber, M.M., Sherr, C.M., and Todaro, G.J.: S-tropic murine type C viruses: Frequency of isolation from continuous cell lines, leukemia virus preparations and normal spleens. Int. J. Cancer 13: 587-598, 1974.

Henderson, I.C., Lieber, M.M. and Todaro, G.J.: Mink cell line MvlLu: Focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology 60, 282-287, 1974.

Todaro, G.J., Sherr, C.J., Benveniste, R.E., Lieber, M.M. and Melnick, J.L.: Type C viruses of baboons: Isolation from normal cell cultures. Cell 2: 55-61, May 1974.

Benveniste, R.E., Heinemann, R., Wilson, G.L., Callahan, R. and Todaro, G.J.: Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. J. Virol. 14(1): 56-67, July 1974.

Sherr, C.J. and Todaro, G.J.: Radioimmunoassay of the major group specific protein of endogenous baboon type C viruses: Relation to the RD-114/CCC group and detection of antigen in normal baboon tissues. Virology. 61: 168-181 (1974).

Lieber, M.M. and Todaro, G.J.: Mammalian type C RNA viruses. In Becker, F.F. (ed.). Cancer: A Comprehensive Treatise. New York, N. Y., Plenum Press. (In press).

Todaro, G.J.: Endogenous viruses in normal and transformed cells. In King, T.J. (ed.). Developmental Aspects of Carcinogenesis and Immunity, New York, N.Y., Academic Press, 1974, pp. 145-158.

Coleman, P.L., Fishman, P.H., Brady, R.O. and Todaro, G.J.: Altered ganglioside biosynthesis in mouse cell cultures following transformation with chemical carcinogens and x irradiation. J. Biol. Chem. (In press).

Sherr, C.J., Benveniste, R.E., Todaro, G.J.: Type C viral expression in primate tissues. Proc. Nat. Acad. Sci. U.S.A. 71: 3721-3725, 1974.

Benveniste, R.E. and Todaro, G.J.: Multiple divergent copies of endogenous type C virogenes in mammalian cells: Nature 252: 170-173, Nov. 1974.

Todaro, G.J., Benveniste, R.E., Callahan, R., Lieber, M., Sherr, C.J.: Endogenous primate and feline type C viruses. In Cold Spring Harbor Symposium on Quantitative Biology XXXIX. The Tumor Viruses. Cold Spring Harbor, N.Y., Cold Spring Harbor Press. Vol. 39, pp. 1159-1168, 1974.

Callahan, R., Benveniste, R.E., Lieber, M.M. and Todaro, G.J.: Nucleic Acid Homology of Murine Type C viral genes. J. Virol. 14: 1394-1403, Dec. 1974.

Benveniste, R.E. and Todaro, G.J.: Evolution of type C viral genes: I. Baboon type C viral nucleic acid as a measure of divergence among primate species. Proc. Nat. Acad. Sci. U.S.A. 71: 4513-4518, 1974.

Benveniste, R.E., Sherr, C.J., Lieber, M.M., Callahan, R. and Todaro, G.J.: Evolution of Primate Type C Viral Genes. Rutgers Symposium, In Gottlieb, A. Arthur (ed.). Fundamental Aspects of Neoplasia, New York, N.Y., Springer-Verlag, 1975.

Sherr, C.J. and Todaro, G.J.: Type C viral antigens in man. I. Antigens Related to Endogenous Primate Virus in Human Tumors. Proc. Soc. Natl. Acad. Sci. U.S.A. 71: 4703-4704, 1974.

Benveniste, R.E. and Todaro, G.J.: Evolution of type C viral genes: II. Inheritance of exogenously acquired viral genes. Nature 252: 456-459, 1974.

Lieber, M., Sherr, C., Potter, M. and Todaro, G.: Isolation of type C viruses from the Asian Feral Mouse Mus Musculus Molossinus. Int. J. Cancer (In press).

Bouillant, A.M.P., Greig, A.S., Lieber, M.M. and Todaro, G.J.: Type C virus production by a continuous line of pig oviduct cells (PFT). J. Gen. Virol. (In press).

Lieber, M.M., Sherr, C.J. and Todaro, G.J.: Transplantable murine tumors release mouse-tropic and xenotropic type C viruses. Int. J. Cancer (In press).

Lieber, M.M., Benveniste, R.E., Sherr, C.J., and Todaro, G.J.: Isolation of a type C virus (FS-1) from the European wildcat (Felis sylvestris). Virology, (In press) 1975.

Sherr, C.J., and Todaro, G.J.: Primate type C virus p30 antigen in human acute leukemia cells. Science 187: 855-857, 1974.

Serial No. ZO1-CP-04822-05-VLL

1. Viral Leukemia and Lymphoma Branch, OADVO, Division of Cancer Cause and Prevention
2. Clinical Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: (A) EBV Studies in Humans and Nonhuman Primates
(B) The Role of RNA Viruses in Human Leukemia, Lymphoma and Breast Cancer

Previous Serial Number: NCI-4811

Principal Investigator: Dr. Paul H. Levine

Other Investigators: None

Cooperating Units:

Inside NIH
Dr. Dharam Ablashi, VLLB, NCI
Dr. Costan W. Berard, LB, NCI
Mr. Roger R. Connelly, BB, NCI
Dr. John Easton, VLLB, NCI
Dr. Ronald B. Herberman, LCBGY, NCI
Dr. Gregory T. O'Conor, DIR., NCI

Outside NIH
Dr. Guy de The, IARC, Lyon, France
Dr. Richard Diamond, NIAID
Dr. Michael Hanna, Litton-Bionetics, Frederick Cancer Research Center
Dr. Clark Heath, CDC, Atlanta, Georgia
Dr. Evan Hersh, University of Texas, Houston, Texas
Dr. John Ho, Queen Elizabeth Hospital, Hong Kong
Litton Bionetics, Bethesda, Maryland
Dr. Nejib Mourali, Institute Salah Azaiz, Tunis, Tunisia

Man Years:

Total	1.0
Professional	1.0
Other	0.0

Project Description

Objectives:

Project A

1. To develop seroepidemiological studies using antibody to EBV-associated antigens to determine whether EBV is etiologically related to human lymphoma.

2. To investigate the role of cellular immunity to EBV as a possible determining factor for the level of various EBV antibodies.

3. To correlate the immune response to EBV-associated antigens and lymphoma-associated antigens in order to determine if there is a relationship between the two.

4. To evaluate the genetic factors controlling the immune response to EBV and susceptibility to lymphoma.

Project B

1. To pursue preliminary studies indicating that there are antigens in human leukemia and breast cancer cells which cross-react with murine leukemia and breast cancer viruses respectively.

2. To evaluate assays for immunity to leukemia and RNA virus-associated antigens in humans and nonhuman primates.

Methods Employed:

Project A

A registry of American Burkitt lymphoma patients (ABLR) was continued in order to obtain a population to compare clinically, epidemiologically, and virologically with the African Burkitt patients. Blood samples were collected from a variety of lymphoma patients, particularly those with American Burkitt's lymphoma and Hodgkin's disease, as well as normal and disease controls. Cell lines known to contain EB virus, fresh tumor biopsies, and lines without evidence of EBV were used as a source of antigen in these studies. Sera were tested for antibody to the viral capsid antigen and the early antigen by immunofluorescence. Lymphocyte cytotoxicity, skin testing, and lymphocyte stimulation assays were employed as tests of cellular immunity. Patients, family members and controls were typed for HL-A antigens to determine possible susceptibility to cancer.

A formalinized Rauscher leukemia virus preparation comparable to that used by Dr. Evan Hersh in human cancer patients was inoculated into mice and monkeys in an attempt to compare the immune response in different animal systems. Cell-mediated immunity was measured by lymphocyte cytotoxicity and lymphocyte stimulation in skin testing. Humoral immunity was measured by a radioimmunoassay.

Membrane extracts from breast cancer tissues and from cell lines derived from breast cancer tissues were inoculated into patients with breast cancer and controls. These in vivo skin test measurements of cell-mediated immunity were correlated with in vitro cell-mediated immunity assays (lymphocyte stimulation, migration inhibition, etc.).

Major Findings:

Project A

Further studies on American Burkitt lymphoma patients identified by the ABLR revealed similarities between American and African cases. Almost 80% of patients with Stage I disease were demonstrated to have long-term survival and no longer required chemotherapy. An excellent response rate was also noted for Stage II patients. The overall long term remission rate was 33%, comparable to that found in Ghana. In the six months between July 1, 1974 and January 1, 1975, 37 new patients have been reported to the American BL Registry and 29 specimens have been obtained from patients and controls.

An EBV producing cell line was obtained from an American BL patient with significant amounts of detectable EBV genome in her tumor.

Twenty-four nasopharyngeal cancer patients from France and Tunisia were skin tested with membrane extracts from cell lines derived from NPC and BL tumors. More than 50% of patients reacted against HKLY-28, a cell line derived from an NPC patient, but a much lower percentage reacted against Raji (a BL-derived line) and cell lines derived from normal individuals, indicating a tumor associated antigen was present in the NPC line. The studies on these patients supported early data obtained in collaboration with Dr. John Ho in Hong Kong. Studies on almost 100 cancer patients with other types of tumors showed no increased reactivity with the NPC cell line, further supporting the specificity of the reaction.

Serologic studies to determine the factors leading to elevated EBV titers in certain groups of individuals continue to demonstrate an apparent genetic factor, with titers remaining high in normal relatives of cancer patients. Further evidence against cell mediated immunity being the major determining factor was provided by a study of patients with disseminated cryptococcosis, a disease of disordered cell-mediated immunity. Laboratory titers in patients with cryptococcosis were no higher than in two groups of controls (normal individuals and patients with familial Mediteranean fever) and anergic cryptococcosis patients had titers that were no higher than normally reactive

cryptococcosis patients. It was demonstrated that each disease required separate analysis when comparison of cell-mediated immunity and EBV titers were made. For example, patients with Hodgkin's disease and solid tumors who were anergic tended to have higher EBV titers than reactive patients with the same disease, whereas patients with nasopharyngeal cancer and non-Hodgkin's lymphoma who had good cell-mediated immunity reactions to skin test antigens had higher EBV titers than those who did not react.

Project B

Mice and monkeys immunized with formalinized Rauscher leukemia virus (RLV) did not demonstrate cell-mediated immune reactivity to RLV antigens, but a significant humoral antibody response was found. Skin testing studies performed in collaboration with Dr. Nejib Mourali, Institute Salah Azaiz, indicated that the breast cancer patients with a fulminant form of breast cancer (poussé evolutive or PEV) were less likely to react to breast cancer related skin test antigens than patients with other forms of breast cancer.

Significance to Biomedical Research and the Program of the Institute:

Project A

The studies in American BL are important since a closer scrutiny of the same disease in geographically distinct areas allow a more careful look at the etiology by separating various factors. If American BL has the same etiology as African BL, it can be evaluated without concern for the role of malaria as a complicating factor. The findings in our studies that some cases of American BL have EBV within the tumor and have high antibodies to EBV while others do not have this EBV pattern parallel findings with African BL. The ability to separate Burkitt's tumor and potentially other lymphomas into diseases associated with different specific virus and tumor associated antigens may lead to identifying different viruses that produce the same morphological disease entity. The American Burkitt Lymphoma Registry is providing detailed information of great importance to oncologists. The geographical distribution and time-space clustering suggest an environmental contribution, perhaps a virus, to the etiology. Characterization of the clinical aspects of the disease is permitting chemotherapists to select drugs which prevent relapse more effectively. The finding of a specific genetic pattern associated with American Burkitt's tumor may explain why a ubiquitous virus would only produce this tumor under rare circumstances in the United States. Studies determining the multiple reasons for high EBV titers serve in conjunction with the various EBV assays to determine the usefulness of EBV serology in a variety of situations. The finding of high EBV titers in normal individuals in cancer families indicate that EBV serology may be used as a test for susceptibility to cancer. Correlation of certain EBV antibodies with clinical stage of the disease allow the use of these particular antibodies in monitoring the effect of conventional therapy as well as immunotherapy. The studies on the relationship of EBV antibodies to cell-mediated immunity are necessary to provide a better understanding of whether or not EBV is oncogenic and if so, how it is activated.

Project B

Comparative studies on breast cancer in Tunisia and the United States will lead to better understanding of factors related to the etiology and control of this tumor. Since the PEV form of breast cancer has been described in developing countries and disappears as the socio-economic conditions improve, the studies in Tunisia provide a unique opportunity to look at the environmental aspects associated with a developing country that clearly affects the incidence and type of a human tumor that may be virus induced. These particular patients with fulminant disease also offer a better opportunity to isolate a human breast cancer virus and to correlate immunity to possible tumor virus associated antigens in a situation that contrasts markedly with that in Europe and the United States.

Comparative studies on immunity to an oncogenic virus in mice, monkeys and humans can demonstrate which model systems, if any, are useful for providing data on the immunologic control of potential oncogenic viruses in humans.

Proposed Course:

Project A

Studies with the American Burkitt Lymphoma Registry will concentrate solely on those cases where adequate material can be obtained for laboratory studies. Because of the variability of pathologic material on many of these cases, it is necessary to separate those with well-fixed tissue sections and unequivocal pathologic features of Burkitt's lymphoma from those cases with atypical features and/or poor histological material for diagnosis. A comparative study of BL patients in Ghana and the United States will be undertaken. Each patient will have imprints evaluated by EBNA assay and longitudinal serum samples; skin tests and in vitro CMI assays will be performed when possible.

The variety of reasons for elevated EBV titers will be pursued in order to determine which assays are of value for immunodiagnosis, monitoring of therapy, and studies of etiology.

Skin testing with a number of EBV and tumor associated antigens and various neoplasms will be continued in order to identify if possible tumor specific and relevant virus related antigens can be used for monitoring conventional therapy and/or for immunotherapy.

Project B

In the next year it is expected that 100 patients with breast cancer will be identified in Tunisia and approximately 20-30 will be studied in the United States. These patients will be followed longitudinally with skin testing, in vitro assays of cell-mediated immunity, clinical examination, and serologic evaluation.

Honors and Awards:

Dr. Levine presented talks on the relationship of EBV to human cancer at a number of medical centers including Walter Reed Army Hospital; Institut Salah Azaiz, Tunis, Tunisia; Antoni Van Leeuwenhoekhuis, Amsterdam, The Netherlands; M.D. Anderson Hospital and Tumor Clinic; American Association for Cancer Research, Southeastern Region, Atlanta Georgia; and Howard University Medical Center, Washington, D.C.

Dr. Levine served as Clinical Assistant Professor of Medicine at Howard University, Freedman's Hospital, Washington, D.C.

Dr. Levine was chairman of the immunology portion of the "Symposium on Treatment of Breast Cancer" held in Teheran, Iran.

Publications:

Levine, P. H. The etiology of Hodgkin's disease. Pathobiol. Ann. 64: 643-660, 1974.

Ablashi, D. V., de Thé, G. B., Easton, J. M., Liabeuf, A., Levine, P. H., and Armstrong, G. R. (Frederick, Lyon, and Bethesda) Antibodies to Epstein-Barr virus (EBV) antigens in sera of American Burkitt lymphoma patients. Biomedicine 20 : 288-293, 1974.

Hersh, E. M., Gutterman, J. U., Mavligit, G., Gschwind, C. R., Freireich, E. J., Levine, P. H., and Plata, E. J. Human immune response to active immunization with Rauscher leukemia virus. 1. Cell-mediated and cell-associated immunity. J. Natl. Cancer Inst. 53 : 317-325, 1974.

Cohen, M. H., Bernstein, A. D., and Levine, P. H. Hematological and serological effects of Rauscher leukemia virus and Epstein-Barr virus on immunosuppressed newborn subhuman primates. Oncology 29 : 353-364, 1974.

Levine, P. H., Gravell, M. The viral etiology of leukemia. Modern Problems in Paediatrics, 16 : 136-164. (In Press).

Herberman, R. B., Char, D., Oldham, R., Levine, P., Leventhal, B. G., McCoy, J. L., Ho, H. C., and Chau, J. C. W. Cell-mediated immunity in human acute leukemia. VI International Symposium on Comparative Leukemia Research (In Press).

Valsamis, M. T., Levine, P. H. Primary intra-cranial Burkitt's lymphoma in an infant. Cancer (In Press).

Levine, P. H., Cho, B. R., Connelly, R. R., Berard, C. W., O'Conor, G. T., Dorfman, R. F., Easton, J. M., DeVita, V. T. The American Burkitt Lymphoma Registry: A progress report. Annals of Internal Med. (In Press).

Levine, P. H., Diamond, R. D., Reisher, J. I. Antibodies to Epstein-Barr virus (EBV) in patients with cryptococcosis. J. Clinical Microbiology (In Press).

Levine, P. H., Herberman, R., McCoy, J., Connelly, R., and Fabrizio, P. Humoral and cellular immunity to EBV and lymphoid cell line antigens in human lymphoma. Second International Symposium on Oncogenesis and Herpesviruses (In Press).

Serial No. Z01-CP-04823-02-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Expression of endogenous mammalian type C viruses

Previous Serial Number: Same

Principal Investigator: Dr. Charles J. Sherr

Other Investigators: Dr. George J. Todaro
Dr. Raoul E. Benveniste
Dr. Robert Callahan

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To study the immunologic relationships of mammalian type C viruses isolated from different species; to determine the extent of expression of endogenous type C virogenes in normal and malignant animal tissues; to define methods for isolating infectious type C viruses from species of animals from which such viruses have not yet been isolated.

Methods Employed:

Components of a wide variety of mammalian type C viruses are purified by standard biochemical techniques and employed for the preparation of specific antisera. Radioimmunoassays are used to test for the presence of viral antigens in extracts of animal tissues and in cultured cell lines. Evidence for type C viral expression is also obtained using biochemical assays for viral polymerases combined with antibody inhibition studies for "typing" enzymes.

In collaboration with other investigators, these assays are supplemented by electron microscopic examination of tissues and cultured cells, molecular hybridization techniques, and a multiplicity of in vitro virologic assays (viral interference, rescue of sarcoma genomes, host range studies).

Major Findings:

Attempts to isolate infectious type C viruses have been directed primarily toward primate cells and tissues. Several independent type C isolates have been obtained from baboon tissues and cultured cell lines. By viral host range and interference, nucleic acid hybridization, radioimmunoassays for viral proteins, and polymerase inhibition studies, the baboon type C viruses have been shown to be distinct from previously described mammalian type C viruses. Sequences related to baboon type C viral RNA have been detected in ten species of Old World monkeys, higher apes, and man, indicating that the baboon viruses are endogenous and have evolved from a primitive ancestor virus present in the primate stock for at least 30 million years, the time of divergence of Old World monkeys and higher apes. Antigens related to the major structural protein (p30) of baboon type C viruses have also been detected in normal baboon, rhesus monkey, and stumptail monkey tissues, and more recently, in several human tumors. These results indicate that humans, like other primates, contain endogenous type C virogenes in their DNA which can, in certain circumstances, be at least partially expressed.

By multiple criteria, the endogenous baboon viruses are related to endogenous viruses of the domestic cat (RD-114/CCC group). Nucleic acid hybridization experiments have demonstrated that only certain members of the genus Felis, all originating from the Mediterranean basin, contain endogenous virogenes related to the RNA genomes of RD-114 and CCC. These findings support the concept that endogenous feline viruses of the RD-114/CCC group originated from an infection of ancestors of the domestic cat by primate type C viruses at a point in recent evolutionary history. Thus, animals of one species can be infected by type C viruses of a second, unrelated group of animals, and acquired virogene sequences can ultimately be perpetuated in the germ line.

The identification of those Felis species which contain virogenes related to the RNAs of known type C viruses has led to the recent isolation of a new type C virus, FS-1, from the European wildcat Felis sylvestris. FS-1 was isolated by cocultivating tissues from a European wildcat with mammalian cell lines, and was obtained free of a contaminating syncytium-forming virus by passage of the mixed virus stock at limiting dilution onto cultured cells which are relatively resistant to infection by feline "foamy" viruses. As predicted from hybridization experiments, FS-1 is closely related by multiple criteria to viruses of the RD-114/CCC group of domestic cat viruses. Its isolation shows that nucleic acid hybridization can be used, at least in certain cases, to predict from which species infectious type C viruses may be isolated.

As a part of a continuing survey of human tissues for the presence of type C viral antigens, we have also focused on a group of type C viruses infectious for primates (SSAV-GALV group). Viruses of this group have been further

studied by radioimmunoassays for p30 proteins and by antibody inhibition experiments performed with the viral reverse transcriptases. These viruses have been shown to be immunologically related to endogenous murine viruses, and sequences related to both SSAV and GALV have been detected in the DNA of normal mice. Our data are consistent with the possibility that this group of viruses, capable of infecting primates and producing tumors, arose from endogenous viruses of rodent, and probably murine, origin. Antigens related to the infectious primate viruses have been detected in peripheral white cells of five human patients with acute leukemia, suggesting that viruses of this group may be associated with neoplasia in man.

Significance to Biomedical Research and the Program of the Institute:

Our studies have confirmed the general validity of in vitro cocultivation methods for the isolation of new classes of mammalian type C viruses. By utilizing rapid and sensitive assays for type C viral antigens, both from previously described and recently isolated viruses, it has been possible to demonstrate that primates, including man, contain antigens in their tissues related to those of both endogenous and infectious primate type C viruses.

Proposed Course:

Experiments are in progress to isolate new type C viruses from other primates with a particular attention to tissues of man. Additional assays for viral components are being developed to aid in the identification of antigens in tissues of primates from which such viruses have not yet been isolated. Further experiments are being designed to study the immunologic relationships of homologous proteins of different groups of type C viruses in an attempt to better define the origins of certain groups of viruses and to evaluate the generality of transpecies infections.

Honors and Awards:

None

Publications:

Benveniste, R.E., Lieber, M.M., Livingston, D.M., Sherr, C.J., Todaro, G.J., and Kalter, S.S.: Infectious type C virus isolated from a baboon placenta. Nature 248: 17-20, 1974.

Sherr, C.J., Lieber, M.M., Benveniste, R.E., and Todaro, G.J.: Endogenous baboon type C virus (M7): Biochemical and immunologic characterization. Virology 58: 492-503, 1974.

Todaro, G.J., Sherr, C.J., Benveniste, R.E., Lieber, M.M., and Melnick, J.L.: Type C viruses of baboons: Isolation from normal cell cultures. Cell 2: 55-61, 1974.

Benveniste, R.E., Sherr, C.J., Lieber, M.M., Callahan, R., and Todaro, G.J.:

Evolution of primate type C viral genes. In Gottlieb, A. Arthur (ed.): Fundamental Aspects of Neoplasia. New York, N.Y., Springer-Verlag, 1975.

Todaro, G.J., Benveniste, R.E., Callahan, R., Lieber, M.M., and Sherr, C.J.: Endogenous primate and feline type C viruses. In Cold Spring Harbor Symposium on Quantitative Biology XXXIX. The Tumor Viruses. Cold Spring Harbor, N.Y., Cold Spring Harbor Press, 1974, Vol. 39, 1159-1168.

Sherr, C.J., and Todaro, G.J.: Radioimmunoassay of the major group specific protein of endogenous baboon type C viruses: Relation to the RD-114/CCC group and detection of antigen in normal baboon tissues. Virology 61: 168-181, 1974.

Sherr, C.J., Benveniste, R.E., and Todaro, G.J.: Type C viral expression in primate tissues. Proc. Nat. Acad. Sci. U.S.A. 71: 3721-3725, 1974.

Sherr, C.J., and Todaro, G.J.: Type C viral antigens in man. I. Antigens related to endogenous primate virus in human tumors. Proc. Nat. Acad. Sci. U.S.A. 71: 4703-4707, 1974.

Lieber, M.M., Sherr, C.J., Potter, M., and Todaro, G.J.: Isolation of type C viruses from the Asian feral mouse Mus musculus molossinus. Int. J. Cancer, in press, 1975.

Lieber, M.M., Sherr, C.J., and Todaro, G.J.: Transplantable murine tumors release mouse-tropic and xenotropic type C viruses. Int. J. Cancer, in press, 1975.

Lieber, M.M., Benveniste, R.E., Sherr, C.J., and Todaro, G.J.: Isolation of a type C virus (FS-1) from the European wildcat (Felis sylvestris). Virology, in press, 1975.

Sherr, C.J., and Todaro, G.J.: Primate type C virus p30 antigen in human acute leukemia cells. Science 187: 855-857, 1974.

Serial No. Z01-CP-04824-06-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies with RNA oncogenic viruses

Previous Serial Number: Same

Principal Investigator: Dr. Adi F. Gazdar

Other Investigators: Dr. Herbert K. Oie
Dr. S. S. Park

Cooperating Units: Inside NIH
Dr. Padman Sarma, VCB, NCI
Dr. Alfred Steinberg, LAR, NIAMD
Dr. Sam Baron, LBV, NIAID
Dr. Ronald Herberman, LCBGY, NCI
Dr. John Minna, IR BG, NHLI
Dr. Richard Lemon, IR BG, NHLI

Outside NIH
Dr. Andrew Abraham, Lankenau Hospital
Philadelphia, Pennsylvania

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

1. To investigate mechanisms of cellular control of type C virus replication.

2. To classify xenotropic strains of murine type C virus by differences in biologic activity and cellular control mechanisms.

3. To establish assay methods for primate tropic type C viruses.

4. To develop new clonal cell lines with biochemical markers useful for cell fusion studies.

5. To establish nonproducer and S+H- derivatives of the wild mouse cell line SC-1.

Methods Employed:

1. Human x mouse hybrid cells are generated by Sendai fusion, selected by the HAT technique, and characterized by isozyme and cytogenetic methods including C banding.

2. Virus replication is monitored by reverse transcriptase assays. MSV pseudotypes of the test viruses are used to determine virus penetration of non-permissive cell lines. Antisera prepared in rabbits are used for neutralization studies. Chronically infected rat cells are used for interference studies.

3. A thioguanine resistant subline of an S+H- BALB/c mouse cell has been developed. The cell provides a highly sensitive assay for murine type C viruses. The line is fused with human and other primate cells, and the hybrids so generated are tested for potential use in assays.

4. Various cell lines are gradually adapted to thioguanine, bromodeoxyuridine or ouabain resistance.

5. The MSV pseudotype of a xenotropic strain of murine type C virus was used to isolate nonproducer clones of SC-1.

Major Findings:

1. Our previous studies have shown that human x mouse hybrid cells support the replication of ecotropic strains of murine type C virus but are resistant to infection with the xenotropic strain AT-124. Experiments were performed to determine whether resistance of the hybrid cells was due to a block at pre- or post-integration level. The thioguanine resistant human cell VA2 was chronically infected with AT-124 virus. The line was fused with freshly liberated NIH Swiss and BALB/c spleen cells, and the human x mouse hybrid cells so generated were isolated and characterized. Eleven of twelve clones released a type C virus indistinguishable from AT-124. All of the clones were either segregating mouse chromosomes or were well balanced. None were segregating human chromosomes. These results, and the previously performed studies, indicate that mouse cellular control of the replication of murine xenotropic virus is exerted at an intracellular pre-integration level. Other studies (some of which are discussed elsewhere in this report) suggest that further cellular control mechanisms also exist.

2. Xenotropic strains of murine type C virus can be subdivided into three subtypes. Xenotropic strains spontaneously isolated or chemically activated from mouse cells replicate efficiently in the rabbit cornea cell line SIRC. Some of these replicate in a large variety of mammalian cells including the human cell VA2. Another subtype fails to replicate in VA2, and their host range is much narrower. Viruses from BALB/c mice fall into this "S" tropic subgroup, while those from NIH Swiss have a wide host range. Neither of these groups replicates in human x mouse hybrid cells or in wild mouse SC-1 cells. In addition, a third subgroup of xenotropic virus has been isolated from certain stocks of FMR viruses. These viruses replicate to high titer in human cells and have an extensive host range. They can be distinguished from other xenotropic strains by their ability to replicate in human x mouse hybrid cells and SC-1 cells. The xenotropic subtypes are not distinguishable by neutralization or interference studies. These results and studies with xenotropic pseudotypes of MSV indicate that most xenotropic strains are inhibited in mouse cells at two levels - at the cell surface as well as intracellularly. SC-1 cells demonstrate only the intracellular block. The xenotropic strains isolated from FMR virus stocks are only inhibited at the cell surface.

3. Clones of human x mouse S+H- hybrid cells have been isolated in HAT medium, but have not been characterized further.

4. Several drug resistant mutants of cells from several mammalian species have been obtained or are in the process of being generated. These include normal as well as MSV transformed mouse, wild mouse, hamster and human cells.

5. MSV transformed nonproducer clones of S+H- cells have been isolated. The clones support the replication of both N- and B-tropic viruses even more efficiently than the parent line, and are suitable for the preparation of high titer MSV pseudotypes.

Significance to Biomedical Research and the Program of the Institute:

Investigations on the nature and mode of action of certain animal type C oncogenic viruses, especially as they affect species other than that of origin will contribute to a more complete understanding of certain human cancers. Indeed, prevention or control of cancer is dependent upon the elucidation of mechanisms by which viruses manipulate species and cellular barriers and further definition of host response to oncogenic agents.

Proposed Course:

1. Similar studies will be performed to determine the control mechanisms of xenotropic viruses from other species.

2. Xenotropic strains isolated from FMR virus stocks will be studied to

determine whether they represent recombinants between FMR virus and the endogenous xenotropic virus present in the cells they are replicating in.

3. Human x mouse S+H- cells will be used to develop assays for primate tropic viruses.

4. Cell lines with biochemical markers will be tested for their usefulness in generating and isolating hybrid cells.

5. S+H- SC-1 cells will be isolated for potential use as assay cells for all mouse ecotropic viruses.

Honors and Awards:

None

Publications:

Gazdar, A.F., Russell, E.K., and Minna, J.D.: Replication of mouse-tropic and xenotropic strains of murine leukemia virus in human x mouse hybrid cells. Proc. Natl. Acad. Sci. USA 30: 2642-2645, 1974.

Herberman, R.B., Aoki, T., Nunn, M., Lavrin, D.H., Soares, N., Gazdar, A., Holden, H., and Chang, K.S.S.: Specificity of ^{51}Cr-release cytotoxicity of lymphocytes immune to murine sarcoma virus. J. Natl. Cancer Inst. 53: 1103-1111, 1974.

Perk, K., Gazdar, A.F., and Russell, E.R.: Studies on a transplantable murine rhabdomyosarcoma. J. Natl. Cancer Inst., in press.

Baron, S., Johnson, H.M., Smith, B.G., and Gazdar, A.F.: Studies of relationship between interferon, antibody and tumor inhibition and enhancement. J. Natl. Cancer Inst., in press.

Serial No. Z01-CP-04825-02-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Physico-chemical studies of viral nucleic acids and enzymes

Previous Serial Number: Same

Principal Investigator: Dr. Daniel K. Haapala

Other Investigators: Dr. Peter J. Fischinger
Dr. Arthur E. Frankel

Cooperating Units: Inside NIH
Dr. Richard Ascione, VB, NCI
Dr. W. Gerry Robey, VB, NCI
Dr. George F. Vande Woude, VB, NCI

Outside NIH
Dr. Jean-Claude Chermann, Institut Pasteur, Paris, France

Man Years:

Total:	2.00
Professional:	1.00
Others:	1.00

Project Description

Objectives:

A. To characterize viral nucleic acids and protein for use as diagnostic tools and to gain insight into the mechanism of viral oncogenesis.

B. To test various antimetabolic compounds which inhibit viral transformation and/or replication in order to obtain products of potential therapeutic and/or heuristic value vis-a-vis the oncogenic process.

Methods Employed:

Standard biochemical, biophysical, and cell culture methods were used. These include ultracentrifugical, chromatographic, enzymological, and nucleic acid hybridization techniques.

Major Findings:

A. Last year we reported the fractionation of reverse transcriptase product DNA from the Moloney sarcoma-leukemia virus complex into three subfractions by sequentional DNA·RNA hybridizations. We have continued this line of experimentation to examine the expression of these different fractions of virus information in producer and nonproducer sarcoma virus transformed cells of various species. This approach represents an attempt to correlate the effects of different regions of the virus genome with the phenotypic change of cells in vitro which we call "transformation". Initial observations have revealed large quantitative differences in viral RNA content of cells which do indeed correlate with the transformed phenotype. Additionally, we have found that certain revertant cells no longer contain a DNA copy of the viral information. Other studies have shown that serial passage of murine sarcoma virus in certain cell lines results in a diminution of the amount of MSV information detectable in the cell DNA. This suggests that not all of the MSV DNA information need be present in the cells in order for transformation to occur. Finally, the use of probes representing the sarcoma specific region of Moloney MSV has permitted us to conclude that the murine sarcoma viruses are genetically heterogeneous. This implies that either the information required for transformation represents only a minute quantity of the sarcoma virus specific information or, alternatively, that very different types of genetic information lead to similar phenotypic changes in the mammalian cell.

B. In collaboration with Dr. Chermann, we have synthesized another inorganic heteropolyanion, antimonium tungstate which is structurally related to silicotungstate whose antiviral activity and antipolymerase activity we reported last year. Antimonium tungstate also functions as a potent inhibitor of type C virus replication in vitro, but unlike silicotungstate, also prevents leukemogenesis in vivo. Further studies have shown that antimonium tungstate is a potent inhibitor of viral reverse transcriptase in vitro. This may explain its antiviral activity.

Significance to Biomedical Research and the Program of the Institute:

The physico-chemical studies of viral nucleic acids and proteins may provide tools with which to detect, characterize and possibly inhibit neoplastic changes of viral etiology.

Proposed Course:

A. Continued studies of virus expression at both the transcriptional translational level will be pursued in order to probe the mechanism of viral oncogenesis. The sequential hybridization technique reported last year allows us to study specific regions of the murine sarcoma virus genome. The discovery that different cell lines contain differing amounts of MSV specific DNA will permit a further fractionation of the viral information and represents an approach to the purification and characterization of MSV

transforming genes at the DNA and RNA level. In addition, the isolation of uncleaved polypeptides containing several MSV gene products (see Vande Woude, et al. Serial No. NCI-4882-1) provides a similar approach at the protein level. These combined studies represent an interdisciplinary approach to elucidating the cell-virus interactions relating to the oncogenic process.

B. The antiviral effects of various chemical compounds will be studied in vivo and in vitro. The purpose of this is twofold: firstly, various inhibitors with different modes of action may provide insight into the mechanisms of viral carcinogenesis, and secondly, compounds may be developed which have therapeutic value. The observation that certain chemicals influence the rate of phenotypic reversion of transformed cells to normal appearance (see Fischinger et al., Serial No. NCI-4826-3) combined with the fact that certain revertant cells have also lost the MSV specific DNA makes it imperative to continue these lines of study because of their obvious clinical implications.

Honors and Awards:

None.

Publications:

None.

Serial No. Z01-CP-04826-03-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer
Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. The nature, mechanisms, and stability of cell transformation induced by murine sarcoma virus (MSV)

B. Nonproductive infection of various mammalian cells with transforming sarcoma genomes: Isolation of endogenous prototype viruses

C. Antigenic and molecular structure-function relationships of purified type C virus components

Previous Serial Number: Same

Principal Investigator: Dr. Peter J. Fischinger

Other Investigators: Dr. Arthur Frankel
Dr. Daniel K. Haapala
Dr. Shigeko Nomura
Dr. Paul T. Peebles
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Dr. Tadao Aoki, VLLB, NCI
Dr. Janet W. Hartley, LVD, NIAID
Dr. C. F. T. Mattern, LVD, NIAID
Dr. W. Gerry Robey, VB, NCI
Dr. George F. Vande Woude, VB, NCI

Outside NIH
Dr. Dani Bolognesi and associates, Duke University, Durham, N.C.
Dr. Luigi Chieco-Bianchi, Univ of Padova, Padova, Italy
Dr. Werner Schäfer and associates, Max-Planck-Institut für Virusforschung, Tubingen, Germany

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

A. To determine the manner of association of the MSV genome with cellular genetic mechanisms; specifically to understand the controls involved in phenotypic or genotypic reversion from the transformed state.

B. To use various pseudotypes of MSV to obtain nonproductively infected heterologous species cells and to rescue from these the endogenous oncornavirus(es) of the species.

C. To isolate substructures and antigens of type C viruses, to clarify the nature of the oncogenic information, and to examine the effect of isolated viral components on the life cycle of oncornaviruses in vitro and in vivo.

Methods Employed:

A. and B. Standard virological assays such as sarcoma virus focus formation, leukemia virus helper assay and focus induction in S+L- cells, viral interference, and virus antigen induction, are being used. Cloning of cells in microtiter dishes and as soft agar colonies with the usual biochemical modes of particle and protein isolation are employed routinely.

C. Biochemical methods such as Sephadex or hydroxyapatite column chromatography, velocity, and isopycnic density gradient centrifugation, radioactive tracer studies, reverse transcriptase activity, gel immunodiffusion, preparative and analytical polyacrylamide gel electrophoresis, and the usual methods of extraction for proteins and nucleic acids are being employed.

Major Findings:

A. Multiple cycles of transformation by MSV and reversion were followed in mouse cells. If the S+L- type Moloney MSV genome was used for both the first and the second transforming cycles, cloned progeny cells showed variable states of MSV rescue, a greater inducibility of endogenous virus by chemicals, a release of an atypical ecotropic MLV, and a failure of being able to revert again. True revertants were also isolated from inbred BALB/c mouse cells transformed by Kirsten MSV. These had a profile of properties different from mouse cells which reverted from the S+L- state. The BALB/c revertants could be retransformed by S+L- type MSV and could revert for a second time.

True revertants have been obtained from sarcoma transformed (S+) cat cells. These had properties of normal parental CCC cells, showed a variable expression of the endogenous CCC genome, and have lost the p30 MuLV marker carried by the sarcoma genome. Human S+L- cells also gave rise to flat variants, but even after multiple cycles of cloning all flat variants proved to be rescuable. Thus only phenotypic reversion was found in human cells.

B. A simple quantitative assay was described in cat S+ cells which was useful for both murine xenotropic oncorna virus (MuX) and feline leukemia viruses (FeLV).

The control of endogenous virus was studied both in cat and mouse cells. Two levels of restriction were seen in each cell system when challenged with the xenotropic MSV pseudotypes (1) cells which were permissive to entry of MSV, (2) cells which prevented the entry of endogenous xenotropic-coated MSV. A cat cell strain was able to shift from one to the other state of restriction.

A surprising result was that the most efficient method of induction of MuX was a coinfection with some but not all ecotropic MuLVs. MuX was thus isolated from outbred and feral mouse cells which were not inducible by chemicals. Potential hybrid viruses which have dual eco- and xenotropic properties are being characterized.

In vivo experiments showed that mouse strains with high incidence of spontaneous leukemia reacted differently to tumorigenesis by MSV coated with an ecotropic MuLV of a type different from the endogenous ecotropic virus. Notable were the longer incubation period and a lack of tumor regression. The MSV found in nonregressing animals has been shown to shift in pseudotype to the natural endogenous virus coat which the host was unable to control.

Kinetics of ecotropic MuLV were examined in synchronized mouse cells to determine the appearance of virus and structural antigens. Cell division was required for both virus release and the appearance of three major viral antigens. The release of xenotropic virus was seen during MuLV infection.

C. The expression of MSV associated MuLV antigens especially p30 was followed in human S+L- cells. The determination of three MuLV antigens was made in S+L- cells of several species in the absence or presence of super-infecting leukemia virus. The expression of these MSV associated antigens was compared in normal mouse cells and Kirsten MSV transformed mouse and rat cells.

The FeLV pseudotype of MSV contained a highly atypical p60 polypeptide. This protein, not found in MSV(MuLV), contained at least MuLV p30 group and interspecies determinants. It could be cleaved enzymatically to lower molecular weight polypeptides which also retained MuLV p30 reactivity. Apparently a cleavage defect exists in the cat cell for MSV.

Expression of MuLV structural antigens on the surface of normal, MSV transformed, and chemically transformed cells was examined. Chemically transformed cells in culture could release ecotropic virus, and contain p10, p30, and gp71 MuLV antigens on the cell surface as determined by cell cytotoxicity. The presence of MuLV antigens was found in mouse cells which did not release MuLV.

The nature and the specificity of MSV specific sequences continues to be examined. Sarcoma specific probes have been purified and are being compared to MuLV and MuX viruses.

A systematic investigation of the neutralizing capacities of antisera produced against isolated MuLV components revealed that cross-neutralization of partially related or even unrelated viruses is possible by some antisera presumably by the interspecies antibody raised against gp71 of Friend MuLV. Except for the antibody raised against the p15 complex of MuLV, antibodies directed against other MuLV structural polypeptides did not neutralize.

Significance to Biomedical Research and the Program of the Institute:

A. Procedures or drugs which would accelerate the rate of reversion from the transformed state has potential therapeutic value. An understanding of cellular controls could be exploited to yield a high frequency of reversion which could be applied to in vivo tumors.

B. Up to now we isolated several endogenous xenotropic oncorna viruses in two species. The extension of analogous procedures to human S+L- cells may lead to the isolation or induction of a human type C virus(es) which may have to be isolated in cells of species other than human.

C. The dissection of the MuLV structure serves as a fundamental model for other mammalian oncogenic viruses with relevance to common structures and functions. Molecular techniques can now be used to differentiate the informational content of the RNA of these various viruses. The specific oncogenic content of the virus can be examined. Hopefully feedback controls exist in virus-infected cells which if introduced into a virus infected cell as specific molecules will result in a termination of virus infections. The nature of the immune response to isolated virus components has to be analysed in reference the state of protection in vivo.

Proposed Course.

A. Further analyses of the reversion process on the molecular level will be made. Multiple cycles of transformation and reversion with various viruses in revertants of several species will be attempted to understand the basic cellular controls of reversion.

B. The isolation of the suspected human endogenous oncornavirus will be attempted. Examination will be made of the possibility of existence of hybrid eco- and xenotropic viruses.

C. To analyze further structure-function relationships of type C virus RNA and antigens. To isolate the informational moiety responsible for cell transformation. To isolate a product of virus growth which could possibly terminate viral replication. To understand the multi-component nature of the in vivo immune response to the virus.

Honors and Awards:

Was an invited guest at a special meeting of 17 participants on the assessment of feline leukemia virus in feline neoplasia on Sept. 2-7, 1974 in Bellagio, Italy.

Presented a seminar at the University of Illinois, Chicago to the Department of Microbiology on Nov. 25, 1974 on the subject of "Reversion of the Malignant Process in Culture".

Publications:

Fischinger, P. J., Nomura, S., and Peebles, P. T.: Control of reversion of MSV transformed cells. In Ito, Y. and Dutcher, R. (Eds.): Comparative Leukemia Research 1973. Tokyo, Japan, University of Tokyo Press, 1974, 475-481.

Peebles, P. T., Fischinger, P. J., and Newman, A. J.: Human amnion and lung tissue culture systems for possible detection and study of human RNA tumor viruses. In Ito, Y. and Dutcher, R. (Eds.): Comparative Leukemia Research, 1973. Tokyo, Japan, University of Tokyo Press, 1974, 363-371.

Fischinger, P. J., Nomura, S., Tuttle-Fuller, N., and Dunn, K. J.: Revertants of mouse cells transformed by murine sarcoma virus. III. Metastable expression of virus functions in revertants retransformed by murine sarcoma virus. Virology 59: 217-229, 1974.

Fischinger, P. J., Blevins, C. S., and Nomura, S.: Simple, quantitative assay for both xenotropic murine leukemia and ecotropic feline leukemia viruses. J. Virol. 14: 177-179, 1974.

Papageorge A. G., Peebles, P. T., Gerwin, B. I., Fischinger, P. J., and Mattern, C. F. T.: Expression of the defective "S+L-" type murine sarcoma virus genome in human amnion and lung cells. J. Natl. Cancer Inst. 52: 1727-1737, 1974.

Nomura, S., and Fischinger, P. J.: Control of reversion in MSV transformed cells after single and multiple transforming cycles. In Robinson, W. S. and Fox, C. F. (Eds.): Mechanisms of Virus Disease. Menlo Park, Calif., W. A. Benjamin, Inc., 1974, pp. 273-285.

Nomura, S., Dunn, K. J., Mattern, C. F. T., Hartley, J. W., and Fischinger, P. J.: Revertants of mouse cells transformed by murine sarcoma virus: flat variants without a rescuable sarcoma virus from a clone of BALB/3T3 transformed by Kirsten MSV. J. Gen. Virol. 25: 207-218, 1974.

Fischinger, P. J., and Nomura, S.: Efficient release of murine xenotropic oncorna virus after murine leukemia virus infection of mouse cells. Virology, in press.

Fischinger, P. J. and Nomura, S.: Alteration of malignancy in cultured cells. Proceedings, XI International Cancer Congress. Amsterdam, Excerpta Medica, in press.

Chieco-Bianchi, L., Colombatti, A., Collavo, D., Sendo, F., Aoki, T., and Fischinger, P. J.: Tumor induction by murine sarcoma virus in AKR and C58 mice: Reduction of tumor regression associated with appearance of Gross leukemia virus pseudotypes. J. Exp. Med. 140: 1162-1179, 1974.

Grant, J. P., Bigner, D. D., Fischinger, P. J., and Bolognesi, D. P.: Expression of murine leukemia virus structural antigens on the surface of chemically induced murine sarcomas. Proc. Natl. Acad. Sci. USA 71: 5037-5041, 1974.

Serial No. Z01-CP-04827-06-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The isolation, purification, and characterization of viral and tumor antigens

Previous Serial Number: Same

Principal Investigator: Dr. Vincent W. Hollis, Jr.

Other Investigators:
Dr. Tadao Aoki
Dr. Ernest J. Plata
Dr. Phyllis B. Brandchaft
Dr. Wm. John Martin

Cooperating Units:

Inside NIH
Dr. Leo Phillips, VLL, NCI

Outside NIH
Dr. Michael B.A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To demonstrate MTV-associated antigen-antibody complexes in the kidney of C3H/He mice.

B. To determine the physical and chemical characteristics of tumor antigens.

C. To test these components to determine their antigenic properties.

D. To analyze which of the purified fractions elicits the production of protective antibody when used for immunization.

E. To develop new and/or adapt known biochemical methods for the isolation and characterization of viruses and/or tumor-associated antigens.

Methods Employed:

A. through E. Standard techniques of differential centrifugation, molecular-sieve (gel) and ion-exchange chromatography, sucrose rate zonal, isopycnic and density gradient ultracentrifugation, and analytical disc-gel electrophoresis are used in the isolation and purification of viral enzymes, antigens, antigen-antibody complexes and tumor specific soluble antigens. The enzyme, RNA-directed DNA polymerase (RDDP) is identified by a standard assay procedure.

Antigen-antibody complexes were dissociated by incubation in low pH buffer and then standard isolation procedures used.

Cell surface antigens of C57BL/6 MBL-2 ascites tumor cells have been solubilized by means of hypertonic salt extraction of cell proteins.

Standard procedures for cell-mediated cytotoxicity inhibition and humoral cytotoxicity inhibition assays have also been utilized.

Major Findings:

A. Specific antibodies to the RDDP of mouse mammary tumor virus, isolated from R-III milk and murine leukemia virus (Rauscher) have been isolated and partially purified from kidney eluates of C3H/He mice. The antibodies were shown to have sedimentation coefficients of 26S to 28S and 5S to 7S on sucrose rate zonal centrifugation. Inactivation with monospecific antisera to various mouse immunoglobulins identified the antibodies as being both IgM and IgG classes. These antibodies only reacted with the RDDP from murine and feline type C viruses but not with the polymerase from avian myeloblastosis virus.

B. Preliminary purification of soluble tumor antigens has been achieved by gel filtration chromatography. Most of the antigenic activity (as determined by inhibition of antibody-dependent lysis of tumor cells) was excluded from the Sephadex G200 gel, although a minor fraction was included in the gel.

C. through E. Solubilized surface antigens are able to inhibit antibody-dependent lysis of tumor cells and also transform normal mouse lymphoid cells into blast (proliferating) cells.

Significance to Biomedical Research and the Program of the Institute:

A. through E. The isolation, purification, and chemical characterization of the antigens and antibodies of murine tumors and oncogenic RNA viruses serve as a model for the study of the etiology and immunology of human

tumors. These procedures will aid in the development of methods for immunological control and prevention of human cancer.

Proposed Course:

This investigator will collaborate in on-going projects whenever possible as a Guest Worker and former member of this Section.

Honors and Awards:

Presented a seminar at Howard University School of Medicine entitled "Naturally occurring antibodies to RNA-dependent DNA polymerase in murine kidney eluates."

Publications:

Phillips, L.A., Park, J.J., and Hollis, V.W. Jr.: Polyriboadenylate sequences at the 3'-termini of ribonucleic acid from mammalian leukemia and sarcoma viruses. Proc. Natl. Acad. Sci. USA 71: 4366-4370, 1974.

Aoki, T., Chieco-Bianchi, L., Plata, E.J., Sendo, F., Hollis, V.W. Jr., and Kudo, T.: Host immune response to virus-induced tumors: Some recent concepts. In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basel, Switzerland, S. Karger, 1974, pp. 23-36.

Aoki, T., Kvedar, J.P., Kudo, T., Plata, E.J., Sendo, F., and Hollis, V.W. Jr.: Enhancement of host immune response to cell surface antigens by the extract of Streptococcus hemolyticus. In Ito, Y. and Dutcher, R.M. (Eds.): Comparative Leukemia Research, 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 237-240.

Serial No. Z01-CP-04828-06-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunology of animal and human tumors, to include studies on viral neoplasia, and detection and characterization of the host's response to tumor associated antigens

Previous Serial Number: Same

Principal Investigator: Dr. Ernest J. Plata

Other Investigators:
Dr. Tadao Aoki
Dr. Phyllis B. Brandchaft
Dr. Vincent W. Hollis, Jr.
Dr. Etsuo Okazaki
Dr. Hideki Kimura

Cooperating Units:

Inside NIH
Dr. Jeffrey Schlom, BCVS, NCI

Outside NIH
Dr. L.M. Boot, Netherlands Cancer Institute, Amsterdam C, The Netherlands
Dr. Evan M. Hersh, M.D. Anderson Hospital and Tumor Institute, Houston, Texas
Dr. Jafa Keydar, Tel-Aviv University, Tel-Aviv, Israel
Dr. Donald Mashburn, Washington Adventist Hospital, Takoma Park, Maryland
Dr. F. Pflugy, Malcolm Grow Memorial Hospital, Andrews Air Force Base, Camp Springs, Maryland
Dr. Dan Moore, Institute for Medical Research, Camden, New Jersey

Man Years:

Total:	2.50
Professional:	1.00
Other:	1.50

Project Description

Objectives:

1. To develop new or to adapt current methods for the detection and characterization of viral or cellular antigens associated with neoplasias.

2. To study the humoral and cellular immune responses of the host to autochthonous and syngeneic tumors.

3. To establish well-characterized cell-culture lines from human and animal tumors, both in vitro and in vivo, to serve as tools in the etiological and immunological studies of the respective tumors.

4. To explore applicable models for immunologic diagnosis, immunotherapy, or possible prophylaxis of tumors.

Methods Employed:

The methods used for the development of objectives 1-4 included mostly standard immunological, animal, biological, biochemical, biophysical, and cell culture techniques. In most cases, these methods were modified sufficiently to render them more sensitive or more efficient for our particular studies.

Major Findings:

1, 2, and 4. Characterization of natural antibodies associated with mammary tumor virus infection.

At least five MTV-associated antigen specificities detected in natural antibodies of MTV$^+$ mouse strains were resolved and partially characterized. The antibodies appeared spontaneously with age and were greatly enhanced when either spontaneous or transplanted mammary tumors developed in mice. This enhancement was not detected in mice with tumors other than mammary tumor. Distinct antibodies were found against thymocytes, MuLV-, MTV-, L-, and fetal antigens. Tumor specific antibodies, if present, were found in minor quantities. The significance of the natural antibodies to the pathogenesis of mammary tumors and the antibodies' relationship to host expression of MTV continue under study.

1. and 3. Detection and characterization of a type C virus associated with L-cells (L-virus).

The L-cell line is a standard cell line derived from normal fibroblasts of a C3H adult mouse. This cell line, while established, has the general characteristics of a non-transformed cell line. It grows as a monolayer and has hardly detectable L-virus production. When L-cells are

inoculated in the syngeneic strain of mice, even into immunosuppressed mice, there is no tumor production. Seeking to develop a subline of L-cells readily useful for cytotoxicity tests, a subline was developed which was altered and grew subsequently in suspension cultures. After 35 passages a special subline was developed which spontaneously produced large quantities of L-virus and now has overt malignant characteristics, i.e., growth in ascites form and tumor production in the strain of origin. The production of this virus is significant because preliminary tests indicate that this may represent a type C, MuLV-reverse transcriptase positive, endogenous virus. Further characterization of this virus, e.g., antigen specificity, host range, etc., is in progress.

3. Establishment of cell culture lines from human tumors to serve as tools in the studies of the respective tumors.

This year we cultured 6 benign and 11 malignant breast tumors. In addition, leukemic cells from 5 different patients were cultured. Our efforts were concentrated in the production of primary and secondary human tumor cell cultures, for use in support of immunological studies that included the examination of primate sera (including human) for evidence of reactivity against those tumors. Cells obtained directly from the tumor specimens were also used in a variety of immunological tests. Three cell lines that were growing well at passage 11-13 and appeared to be good candidates for established cell lines were stored frozen to await further study.

4. Active immunization of syngeneic mice against mammary tumors.

Immunization of syngeneic mice with mammary tumor was attempted with tumor extracts, X-irradiated or formalin-fixed tumor cells, and by complete tumor removal of growing transplanted tumors. Repeated attempts using a variety of protocols failed to induce significant antibody production with all but one of the immunogens. Treatment of C3H/He mice with formalin-fixed C3H/He mammary tumor cells consistently produced antibodies of several specificities, including some which appear to be tumor specific. The nonspecific antibodies produced appear to be similar to the natural antibodies described previously.

1. to 4. Administrative role in extramural contract program of collaborative research.

In addition to the research activities generally described, approximately one half of this investigator's efforts have been spent in the administration of the Virus Cancer Program as Executive Secretary of the Breast Cancer Virus Segment and as Project Officer on several contracts.

Significance to Biomedical Research and the Program of the Institute:

The characterization of natural antibodies associated with murine mammary tumors is significant because it sheds light on the inter-relationship of virus expression, malignant transformation, and the mechanism of the host immune response to tumors. Specifically these studies and those on the concomitant antigens can effectively aid to (a) devise methods for early diagnosis of an oncogenic virus infection and tumor onset; (b) develop a better understanding of the pathogenic relationships among the host, an oncogenic virus, and a solid tumor; (c) analyze epidemiological and genetic influences on tumor susceptibility; and (d) serve as a powerful model for the clinical status of the tumor host, thus serving to evaluate potential methods of therapy. This model in C3H/He mice could serve to develop similar methodology likewise useful in the case of human solid tumors. In addition, this finding provides another example of immunity rather than immunological tolerance to antigens possibly acquired congenitally. The elevation of such antibody titers in the presence of mammary tumors (in our model system) may give further clues concerning the dynamics of tumor-host equilibrium.

The active immunization of C3H/He mice against their mammary tumor shows a model of immunity elicited preferentially by formalin-fixation of antigens. Further study is required urgently to determine the applicability of this finding to other tumors and systems where an immune response has been nearly impossible to elicit, leading to conclusions of irreversible tolerance to that given tumor. The finding and production of L-virus will be of great aid to the program if we can confirm its nature as an endogenous virus. Clarification of the role and function of type C endogenous viruses are among the high priority items of the Cancer Program.

Proposed Course:

The goals of this project for the next fiscal year are:

1. To advance significantly in the study of soluble antigens associated with MTV to determine the incidence, diagnostic value and immunologic implications for solid tumors.

2. To continue the study of the relationship of naturally occurring antibodies to the host susceptibility, incidence of these antibodies and immunogenetic influence on tumor incidence.

3. To identify one or more tumor or viral specific antigens from human human breast tumors.

4. To study the question of whether animal oncogenic viruses play a discernible role in human leukemia or breast cancer.

Awards and Honors:

Chairmanship of Session on Tumor Immunology at the 1975 Annual Meeting of the American Society for Microbiology, New York.

Publications:

Aoki, T., Chieco-Bianchi, L., Plata, E.J., Sendo, F., Hollis, V.W. Jr., and Kudo, T.: Host immune response to virus-induced tumors: Some recent concepts. In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basal, Switzerland, S. Karger, 1974, pp. 23-36.

Aoki, T., Kvedar, J.P., Kudo, T., Plata, E.J., Sendo, F., and Hollis, V.W. Jr.: Enhancement of host immune response to cell surface antigens by the extract of Streptococcus hemolyticus. In Ito, Y. and Dutcher, R.M. (Eds.): Comparative Leukemia Research, 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 237-240.

Hersh, E.M., Gutterman, J.U., Mavligit, G., Gschwind, C.R., Freireich, E.J., Levine, P.H., and Plata, E.J.: Human immune response to active immunization with Rauscher leukemia virus. I. Cell-mediated and cell-associated immunity. J. Natl. Cancer Inst. 53: 317-325, 1974.

Serial No. Z01-CP-04829-02-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The molecular structure and expression of the endogenous type C viral genome

Previous Serial Number: Same

Principal Investigator: Dr. Robert Callahan

Other Investigators: Dr. Raoul E. Benveniste
Dr. Joseph E. DeLarco
Dr. Charles J. Sherr
Dr. George J. Todaro

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To determine the degree of nucleic acid homology between the different host range classes of murine endogenous type C viruses, the nature of the differences between the viral genomes, and whether it is possible that the different host range classes of murine type C virus have a common origin; to determine whether there is a correlation between the virogene nucleic acid sequences contained in the cellular genome of various inbred strains of mice (and feral strains of Mus musculus) and the frequency at which these mouse tropic viruses can be isolated from these strains.

Methods Employed:

Viruses were isolated by cocultivation of organs from different strains of mice with permissive tissue culture cell lines; or from murine cell lines which spontaneously release or can be induced to release endogenous type C

virus. Molecular hybridization is employed to fractionate and compare viral genomes. Biochemical assays for expression of the viral genome in vivo include those for gs antigen, viral reverse transcriptase, and viral RNA synthesis.

Major Findings:

On the basis of DNA:RNA hybridization data obtained during the past year, the following observations have emerged. First, the murine endogenous type C virus can be classified in three groups which parallel their differences in host range and immunologic properties of the viral protein p12. Thus, the mouse (N- and B-) tropic viruses are more related to each other (by greater than 95%) than they are to the BALB/c or NIH Swiss xenotropic virus (X-tropic, 60% and 40%, respectively). A similar relationship exists between the viral genomes of laboratory strain leukemia viruses (Kirsten, Rauscher, and Moloney strains) and the BALB/c endogenous type C viruses, i.e., they are more related to the mouse tropic viral sequences than to the X-tropic viral sequences. This is particularly evident in the case of the Kirsten type C viral genome which is greater than 95% homologous to that of the BALB/c N-tropic endogenous type virus. Second, the murine X-tropic virus can be divided into at least two subclasses depending upon their relative homology to the BALB/c and NZB/BINJ X-tropic type C virus. The viral genome of the X-tropic virus isolated from the inbred strains AKR/J, CBA/J, DBA/2J, C57BL/J, and C58/J and those isolated from the Asian feral subspecies M. musculus molossinus and M. musculus castaneus are highly homologous (greater than 85%) to the BALB/c viral genome. This class of X-tropic virus is significantly less related to the NZB/BINJ and NIH Swiss X-tropic viruses (60% and 30-40%, respectively). Third, the thermal stability of the hybrids formed between members of the different classes suggest that the partial homology obtained results from the accumulation of small segments of base substitutions throughout the genome rather than by the retention of a common identical long segment of base sequences coupled to sequences completely unique to each virus class. Fourth, inbred strains of mice (AKR/J, BALB/c, and NIH Swiss) and feral subspecies of Mus musculus contain in their cellular genome type C virogene sequences in multiple (6 to 7 copies per haploid genome) copies that are related by varying degrees to the BALB/c N- and X-tropic viral genomes.

A model which accommodates the presently available data for the major subclasses of endogenous murine type C viruses proposes that all murine endogenous type C virogenes developed from a common ancestor (Vo) through gene duplication and subsequent mutational events. A common ancestor would account for the partial homology of all murine type C viral genomes. The major source of virogene diversity between inbred mouse strains can probably be attributed to what Mayr has called the "founder principal." This designates the evolutionary consequences of "...the establishment of a new population carrying only a small fraction of the total genetic variation of the parental population." We propose that the two subclasses of X-tropic virogenes diverged from the ancestral virogene, whereas the mouse tropic virogene sequences evolved from the BALB/c group of xenotropic viruses. This is supported by two lines of evidence. First, all strains tested from which mouse tropic viruses have been recovered possess X-tropic viruses which are highly related to the BALB/c

X-tropic virus. Those strains from which the NZB related type C virus have been obtained (NZB and NIH Swiss) have not yielded mouse tropic viruses despite extensive attempts to isolate them. Second, by nucleic acid hybridization criteria, the viral genomes of the mouse tropic class and the BALB/c related subclass of X-tropic virus share a greater degree of sequence homology than either does to the NZB related subclass of X-tropic viruses. This model, however, does not apply to laboratory strain murine leukemia virus stocks which have been extensively passaged with the resulting opportunities for rapid genome mutation and recombination.

Significance to Biomedical Research and the Program of the Institute:

Since mice are not unusual in possessing multiple copies of related endogenous type C virogene sequences (multiple related virogene copies are also present in the cellular DNA of rat, pig, domestic cat, and baboon species), the murine system provides a unique model to study the nature of the different endogenous virogenes of a species and how they evolve. Second, the murine xenotropic viruses due to their ability to grow on primate and other mammalian cell lines may be relevant in the consideration of the origin of primate and type C viruses of other species. In this regard, work to classify and characterize members of the murine xenotropic class of endogenous type C viruses is highly relevant to other projects in progress in this and other laboratories.

Proposed Course:

The same strains of mice which possess the common mouse tropic virogene sequences (AKR/J, C58/J, BALB/c, DBA/2J, C57BL/6J) also have the subclass of X-tropic virogenes which are highly related to BALB/c X-tropic viral sequences. In contrast, the NIH Swiss and NZB/BINJ strains of mice from which no mouse tropic viruses have been isolated contain X-tropic virogene sequences which constitute a separate subclass. This interrelationship suggests that mouse tropic and BALB/c related X-tropic virogenes may be inherited in a coordinate fashion. The possibility that these two subclasses of murine endogenous virogenes are linked is testable by genetic methods, as is the possibility that the BALB/c and NZB related subclasses of murine X-tropic virogenes are still allelic.

One major difference between NIH Swiss and NZB/BINJ with regard to their endogenous type C viruses is the degree of control they exert over the expression of these virogenes. Thus, NZB mice produce high levels of xenotropic virus throughout their life whereas there is little expression of these virogenes in the NIH Swiss strain. Work is currently under way to determine the nature of the apparent regulatory defect in NZB mice.

Honors and Awards:

None

Publications:

Benveniste, R.E., Heinemann, R., Wilson, G.L., Callahan, R., and Todaro, G.J.: Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. J. Virol. 14: 56-67, 1974.

Callahan, R., Benveniste, R.E., Lieber, M.M., and Todaro, G.J.: Nucleic acid homology of murine type C viral genes. J. Virol. 14: 1394-1402, 1974.

Benveniste, R.E., Sherr, C.J., Lieber, M.M., Callahan, R., and Todaro, G.J.: Evolution of primate type C viral genes. In Gottlieb, A. Arthur (ed.): Fundamental Aspects of Neoplasia. New York, N.Y., Springer-Verlag, 1975.

Todaro, G.J., Benveniste, R.E., Callahan, R., Lieber, M.M., and Sherr, C.J.: Endogenous primate and feline type C viruses. In Cold Spring Harbor Symposium on Quantitative Biology XXXIX. Cold Spring Harbor, N.Y., Cold Spring Harbor Press, 1975.

Callahan, R., Lieber, M.M., and Todaro, G.J.: Nucleic acid homology of murine xenotropic type C viruses. J. Virol. (In press).

Serial No. Z01-CP-04830-03-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Transcriptional control of endogenous RNA tumor viruses.

Previous Serial Number: Same

Principal Investigator: Dr. Andrew Y. Silverman

Other Investigators: Dr. Edward M. Scolnick

Cooperating Units: Inside NIH
Dr. Wade Parks, VC, DCCP, NCI

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia and Rockville, Maryland

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description

Objectives:

The work of the past year has been to develop an assay for measuring the number of initiation sites on template active chromatin for mammalian RNA polymerase II. Template active chromatin is still restricted in template activity compared to free DNA. The question being asked is whether this restriction is at initiation or elongation.

Methods Employed:

Template active chromatin has been purified by shearing and sedimentation in steep glycerol gradients. RNA polymerase II has been purified by DEAE, Sephadex, and Phosphocellulose column chromatography. A protein factor stimulating RNA polymerase II has been purified by phosphocellulose and DEAE chromatography. An initiation assay, using the polyanion, heparin has been

developed which inhibits free RNA polymerase II but not RNA polymerase II prebound to template active chromatin or DNA.

Major Findings:

The major finding thus far is that the protein stimulatory factor stimulates elongation not initiation. The restriction for chromatin transcription by RNA polymerase II is at elongation. This is in contrast to earlier results with E. coli RNA polymerase which seems to be restricted in initiation. The reasons for the differences with the two enzymes are being investigated.

Significance to Biomedical Research and the Program of the Institute:

The mechanism by which certain tumor virus genes are expressed in some cells and repressed in others remains unknown but of primary importance. The nature of the restriction to transcription of certain genes but not others in chromatin will help to elucidate this question. These findings may be necessary in eventually designing ways to control tumor virus expression, and thus the induction of tumors by RNA tumor viruses.

Proposed Course:

The next year will be devoted to measuring the specific viral gene transcripts from chromatin after blocking reinitiation. Different in vitro conditions will be employed. Attempts will be made to identify specific nonhistone or histone proteins controlling type C and type B virus transcription.

Honors and Awards:

None

Publications:

Howk, R.S., Anisowicz, A., Silverman, A.Y., Parks, W.P. and Scolnick, E.M.: Distribution of murine type B and type C viral nucleic acid sequences in template active and template inactive chromatin. Cell, in press.

Serial No. ZO1-CP-04831-04-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on factors affecting local and general growth of transplants of reticulum cell sarcomas type B in BALB/c mice.

Previous Serial Number: Same

Principal Investigator: Dr. Ruth Merwin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To continue studies on type B reticulum cell sarcomas that arise spontaneously in old BALB/c mice. Previous studies demonstrated that immunity greatly increased the amount of local as compared to metastatic growth of transplants of one of these tumors. Work this year focused on the extent to which immunity affected the local and generalized growth of other spontaneous type B reticulum cell tumors.

Methods Employed:

A method of immunizing mice with heat treated cells instead of X-irradiated cells, as reported by others, was modified for use with these tumors. Cells were heated for 10 to 50 minutes at 46°C until 3 out of 4 stained with trypan blue. Inoculation of these cells induced immunity similar to that caused by inoculation of X-irradiated cells.

Major Findings:

A challenge dose of 1×10^5 cells of one tumor failed to grow in mice immunized against the same tumor. Of the other 5 tumors tested, 3 showed a lengthening of the latent period by 14 to over 80 days after challenge and 2 showed no effect. None cross reacted.

The most antigenic tumor grew locally with no metastases; neither ATS treatment nor X-irradiation of the transplant host caused metastases to develop. Two of the weakly antigenic tumors, both of which normally had no local growth, produced slight local growth in some immunized mice. Although immunity caused local growth of one tumor studied previously it appears that for some of these type B reticulum cell tumors, immunity has little effect on the amount of local as compared to metastatic growth. An increase in the amount of local growth occurred spontaneously in several tumor transplant lines in the early transplant generations.

After February 1974 young mice of the original type BALB/cCr, called Cr here, were not available. The new mice, called Crf, were descendants of a few cesarean derived Cr mice foster nursed on Swiss mice. Tumors arising in Cr mice grew in only an occasional Crf mouse. It was thought that this failure of tumors to grow might be due to genetic drift together with a freeing of the mice from debilitating viruses by foster nursing on Swiss mice. However, it has been found that the Crf mice are Swiss, or at least partly Swiss. Thyroid grafts from Crf mice disintegrated in 3 to 5 days after being placed in BALB/cAn mice previously inoculated with Swiss tissue. Control Crf thyroid grafts were well vascularized and in good condition even at 10 days after grafting. Most of the work reported here was done with transplants of spontaneous BALB/cCr tumors into BALB/cAn mice. No evidence of genetic drift was detected.

ATS prevented local tumor growth in 2 tumors when both tumor cells and ATS were inoculated subcutaneously. When both were inoculated intraperitoneally or when they were inoculated at different sites the latent periods were lengthened by one or two weeks. The same tumors that ATS prevented from growing locally in syngeneic mice grew only in the presence of ATS in the allogeneic Crf (Swiss) mice. This observation suggests that the action of the ATS is not direct upon the tumor cells.

Of 18 premary tumors transplanted to mice 8 months or more of age and to young mice less than 2 months of age, 15 grew in the old and 5 in the young within 4 months of inoculation and relatively few grew thereafter. Those positive in the young hosts were the ones with the shortest latent period, that is less than 2 months, in old hosts. In the second generation of transplant almost all tumors grew in weanling mice.

Significance to Biomedical Research and the Program of the Institute:

The type B reticulum cell neoplasm in BALB/c mice merits study because it is the animal tumor most like human Hodgkin's disease. A study of late growth of transplants of the reticulum cell tumors may contribute to an understanding of late recurrences of tumors in humans after chemotherapy.

Proposed Course:

To continue studies on the reticulum cell neoplasms including observations on the growth of transplants after long latent periods and including observations on the correlations of tumor gross pathology in the primary host with the amount of local growth in the derived tumor lines. To study in this varied group of reticulum cell neoplasms the relationship of local and general growth to the degree of differentiation of tumor cells and to the presence of B and T cells and macrophages.

To investigate the induction of sarcomas adjacent to plastic or other materials placed subcutaneously or intraperitoneally in mice. Reports in the literature suggest that a poor vascular supply does not contribute to the induction of these tumors but observations made in this laboratory indicate that a poor vascular supply should still be considered as a possible causative agent.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04832-01-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and characterization of tumor virus-induced surface antigens: Use of these solubilized antigens for in vitro sensitization of normal lymphoid cells

Previous Serial Number: NCI-4872

Principal Investigator: Dr. Phyllis B. Brandchaft

Other Investigators: Dr. Tadao Aoki
Dr. Vincent W. Hollis, Jr.
Dr. Hideki Kimura

Cooperating Units: None

Man Years:

Total:	2.25
Professional:	1.00
Other:	1.25

Project Description

Objectives:

1. To isolate, purify, and characterize cell surface antigens from Moloney virus-induced mouse leukemia cells.

2. To effect the in vitro immunization of normal mouse spleen cells by solubilized Moloney virus-associated and malignant cell specific surface antigens.

3. To study the biological activity of lymphocytes stimulated by crude soluble antigens and purified antigenic components.

Methods Employed:

1. through 3. Inhibition of the complement-dependent ^{51}Cr release cytotoxicity assay was used to measure soluble antigen activity. The uptake of ^{3}H-thymidine was used to assess antigen-induced proliferation of

normal lymphoid cells. The maturation of sensitized lymphocytes into cytotoxic effector cells was determined by the cell-mediated cytotoxicity assay. Soluble antigens were obtained from Moloney virus-induced leukemia cells using hypertonic salt (3M-KCl) extraction. Gel filtration, chromatography and other commonly used biochemical procedures were used for the isolation and purification of macromolecules.

Major Findings:

1. and 2. Most of the time so far spent on this project has involved establishing optimum conditions for the preparation and assay of stable soluble antigens and the culturing of normal lymphocytes. Some preliminary results in each area have been obtained.

Antigens from Moloney virus-induced leukemia cells were isolated in soluble form. Antigenic activity was retained by storage at -70°C for at least 4 months. We are presently investigating methods for increasing the specific activity of the preparations. The soluble antigens were partially purified by Sephadex G-200 column chromatography. Most of the activity was eluted with the void volume, although a smaller proportion was included in the gel.

2. and 3. Normal C57BL/6 spleen cells cultured in vitro (at 37°C, 10% CO_2, continuous rocking) with 0.1 mg of the crude soluble antigen preparation showed a peak of ^{3}H-thymidine incorporation after 3 days incubation. Incubation with formalin-fixed antigens gave an identical pattern of thymidine uptake. Cultures sensitized with soluble antigens for 6-8 days showed some cell-mediated cytotoxic activity on ^{51}Cr-labeled Moloney virus-induced leukemia cells.

Significance to Biomedical Research and the Program of the Institute:

1. through 3. Cancer patients cannot usually mount an effective immune response to their own tumors. Therefore, the adoptive transfer of immunity with lymphocytes sensitized in vitro to tumor antigens may be an effective method to suppress the tumor in vivo. Soluble antigens represent harmless, yet potent immunogens.

In addition, the use of purified antigenic components will allow us to evaluate the immune response to the individual antigens present on the tumor cell surface.

Proposed Course:

1. To isolate purified antigenic components from solubilized murine leukemia cells.

2. and 3. To evaluate the immune response in vitro and in vivo by lymphoid cells sensitized in vitro to soluble antigens.

Honors and Awards:

Appointment to Adjunct Professor, 1974-1975; Biology Department, Antioch College, Yellow Springs, Ohio.

Publications:

Brandchaft, P.B. and Boone, C.W.: Increase in Gross(G) antigen sites on the surface of AKR virus-induced rat lymphoma cells after treatment with trypsin. J. Immunol. 113: 94-102, 1974.

Brandchaft, P.B. and Boone, C.W.: Extraction of Gross(G) surface antigens from AKR mouse lymphoma cells with 3M potassium chloride. J. Natl. Cancer Inst. 53: 1079-1083, 1974.

Serial No. ZO1-CP-04833-01-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause
   and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: (A) Characterization and isolation of virus-induced tumor surface antigens. (B) Utilization of formalin-fixed virus-induced tumor cells for immunization.

Previous Serial Number: None

Principal Investigator: Dr. Etsuo Okazaki

Other Investigators: Dr. Tadao Aoki
Dr. Ernest J. Plata
Dr. Phyllis Brandchaft
Dr. Hideki Kimura

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To distinguish type C virus-induced cell surface antigens from tumor-specific cell surface antigens acquired by the cellular genome through malignant transformation, and to characterize these antigens.

B. To determine the enhanced immunogenicity of virus-induced tumors using formalin-fixed tumor cells.

Methods Employed:

A. and B. Establishment of new transplantable AKR leukemias, ^{51}Cr-release cytotoxicity tests, indirect immunofluorescence microscopy, various cross-absorptions of antisera, and formalin-fixation of tumor cells (0.1% formalin, 4°C, 7 days).

Major Findings:

A. Antigens on the cell surface of 5 newly established transplanted AKR spontaneous leukemias (tentatively designated SKL-1,2,3,4, and 5) have been demonstrated by indirect immunofluorescence microscopy using hyper-immune serum of (C57BL/6 x C3Hf/He)F_1 mice against formalin-fixed AKR leukemia cells. The positive reaction with viable cells of these leukemia lines remained after absorptions with thymocytes, spleen cells, and lymph node lymphocytes of 1- to 2-month-old non-leukemic AKR mice. However, the absorbed antiserum showed a negative reaction with normal AKR thymocytes in contrast to the positive reaction of unabsorbed antiserum. These results strongly indicate that the cells of these AKR leukemias carry specific surface antigens distinguishable from both virus-associated surface antigens and alloantigens. Further studies to determine whether individual AKR leukemia lines contain different antigen specificities are in progress using cross-absorption tests.

B. High titer antisera were consistently produced by syngeneic and/or allogeneic immunization with formalin-fixed virus-induced leukemia cells (BALB/c Moloney leukemia LSTRA and C57BL/6 Moloney leukemia MBL-2). Examinations of the time of antibody appearance, antibody titers, and specificity have shown that formalin treatment results in significantly enhanced humoral antibody production in certain antigen systems in comparison with viable cells in immunizations. Further characterization of the effect of formalin treatment on cell surface antigens and on humoral and/or cell-mediated antibody production are also in progress.

Significance to Biomedical Research and the Program of the Institute:

A. Although mouse mammary tumor-specific cell surface antigens acquired by the cellular genome through malignant transformation have been distinguished from mammary tumor virus-associated surface antigens, no evidence for the presence of such antigens as the former has been reported in type C virus-induced tumors. Consequently, an important model for the development of an improved method of cancer diagnosis and immunotherapy will be provided since somatic cells are also infected with type C viruses and might be damaged by antibodies against virus-associated surface antigens. This study also contributes to a better understanding of the basic biology and virology of oncogenic virus-host interaction.

B. Successful and more effective immunization with formalin-fixed cells in syngeneic systems would bring in an easier approach to tumor immunology by supplying competent antisera and opening a way to possible immunotherapy. In particular, when virus-induced tumor cells are used for immunotherapy of patients, we can ethically avoid the biohazard risk of inducing new tumors in humans by virus.

Proposed Course:

A. To study differences in the nature and specificity of surface antigens between virus-infected nonmalignant and virus-induced malignant cells.

B. To analyze the mechanism of the effect of formalin-fixation on surface antigens of virus-induced tumor cells and on the characteristics of the antibodies produced.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04834-06-VLL

1. Viral Leukemia and Lymphoma Branch, OADVO, Division of Cancer Cause and Prevention
2. Primate Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Herpesvirus saimiri and Epstein-Barr Virus

B. Further propagation and characterization of Rauscher leukemia virus in human and simian cells

C. International activities

Previous Serial Number: Same

Principal Investigator: Dr. Dharam V. Ablashi

Other Investigators: Dr. John M. Easton

Cooperating Units: Inside NIH
Dr. V. Zeve, VO, NCI
Dr. H.K. Oie, VLL, NCI
Dr. P. Levine, VLL, NCI

Outside NIH
Dr. G.B. de Thé and Mrs. M.L. Didier, International Agency for Research on Cancer, Lyon, France
Dr. C. Jasmin, Institut de Cancerologie et d'Immunogenetique, Villejuif, France
Dr. H. zur Hausen and Dr. B. Fleckenstein, Institut fur Klinische Virologie der Universitat Erlangen-Nuremberg, Germany
Dr. P. Periman, George Washington University, Washington, D.C.
Dr. H. Rabin, Litton Bionetics Research Laboratories, Kensington, Maryland
Dr. L.J. Charamella, Litton Bionetics, Inc. Frederick, Maryland
Dr. P. Ebbesen, Institute Medical Microbiology, University of Copenhagen, Denmark
Dr. D. Twardzik and Dr. H. Aaslestad, Litton Bionetics, Inc., Frederick, Maryland

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

A. & B. The oncogenic potential of Epstein-Barr Virus (EBV) in nonhuman primates and the presence of EBV genome in epithelial cells in nasopharyngeal carcinoma have provided further evidence for the association of EBV and human cancer. Moreover, the oncogenic potential of Herpesvirus saimiri (HVS) and Herpesvirus ateles (HVA) relates oncogenic herpesviruses to lymphoproliferative neoplasia. We hope to continue immunological, epidemiological, biological, biochemical, and pathological studies in order to understand the role of herpesviruses in cancer in man and animals.

C. The search for an oncogenic type C virus in human neoplasia is being maintained. The possibility that a known animal type C can be oncogenic in another species after its adaptation in vitro cannot be overlooked. The objective of the project is to explore such possibilities employing murine Rauscher leukemia virus after its propagation in simian cells.

D. International collaboration in the Cancer Program has a vital role in providing a mutual exchange of scientists, reagents, and information. This activity, after its initiation last year, is continuing and being extended.

Methods Employed:

A., B., & C. Primary and continuous cell lines of human and nonhuman origin were used for virus isolation, biological, biochemical assays and in vivo experiments. Tumors of human and nonhuman origin were employed for the establishment of cell cultures. For in vivo experiments, marmoset, owl monkey and mice were used. In some experiments, mouse and rat cell cultures were employed.

Major Findings:

A. 1. Twenty-five sera from 21 patients with American Burkitt lymphoma were assayed for antibodies to four different EBV antigens: VCA (viral capsid antigen), CF (particulate complement fixing antigen), EA (early antigen) (15 sera only), and NA (nuclear antigen). The American Burkitt sera had higher geometric mean titers (GMT) than the controls to all four antigens. In the case of NA, the GMT of the American Burkitt sera was 59, while that of the ten controls was 1.5. Although 11 of 25 of the American Burkitt sera had negative titers for one or more antibodies, none of 15 sera were negative for all four antibodies. Thus, the data suggest a possible relationship between EBV and American Burkitt lymphoma. Some biopsies of

American Burkitt lymphoma (AmBL) tumors have been negative for the presence of EBV genome. The AmBL B-lymphoblastoid cell line (NAB-1) grows rapidly, possesses high soluble complement fixing antigen levels, seems better for EBV-NA testing, and is equal to Raji cells for inducibility of EA. Collaborative studies with Dr. zur Hausen have shown that this cell line contains approximately the same number of EBV genomes as Raji cells.

2. Two soluble (S) complement fixing (CF) EBV antigen complexes were isolated from ^{14}C-labeled cell cultures of P_3HR1 (EBV producer). The first antigen complex is further separable into two components with molecular weights of 40,000 and 200,000 daltons. The second antigen component is a single protein with a molecular weight of 70,000 daltons. The crude S antigen is stable at -70°C at least for three months and is partially resistant to heat (56°C for one hour). The CF activity is readily destroyed by treatment with trypsin, acetone, chloroform, and pronase. Lipase and phospholipase did not completely destroy the CF activity of the S antigen but reduced it significantly.

3. Soluble antigen from Raji cells containing 7 mg/ml of protein and with a CF titer of 1:8 (with an NPC serum) was used by Dr. Periman for in vitro leukocyte migration tests employing lymphocytes of lymphoma patients with histories of infectious mononucleosis (IM), normal medical students, and lymphoma patients without a history of IM. Results showed that $\geq$ 20 percent inhibition was observed with the group of lymphoma patients with an IM history and not with normal medical students. An occasional lymphoma patient with no IM history gave $\leq$ 5 percent inhibition, thus, pointing out the specificity of reactivity of the antigen when there was a history of EBV involvement. Further experiments will utilize purified components of S antigen. These studies also will be related to the titer of antibodies to EBV antigens in the sera of the patients.

4. A comparison of DNA-dependent DNA polymerase activities in producer (P_3HR1) and nonproducer (Raji) EBV cell systems and in an American Burkitt's cell system was made. All cell systems contained a nuclear (4S) and cytoplasmic (6-8S) DNA polymerase; a minor 10S-DNA-dependent-DNA polymerase activity was also seen in high VCA P_3HR1 cell cultures. All cultures demonstrated a high salt manganese dependent RNA-dependent DNA polymerase activity corresponding to cellular DNA polymerase III. Minimal terminal deoxynucleotide transferase activity was also demonstrated in all cells examined.

5. The ammonium salt of the heteropolyanion 5-tungsto-2-antimoniate (HPA-23) was shown by Dr. Jasmin to protect mice against Friend and Plasma variant induced leukemia as well as delayed development of sarcoma induction by MSV. It was also shown that HPA-23 possesses a potent anti-viral activity against various RNA and DNA viruses. A simian cell culture (MEST) chronically infected with HVS, when treated with HPA-23 showed enhancement of HVS early and late antigens and infectious HVS.

HPA-23 inhibited both DNA polymerase I and II from P_3HR1 cells, but inhibition of DNA polymerase I was more pronounced. The effect of HPA-23 on oncogenic herpesviruses may be useful in increasing the production of transforming and non-transforming EBV, HVS and their antigens.

6. Both the MLC cell line (T lymphocytes from a marmoset developing HVS lymphoma) and MEST-HVS cells were further characterized. These were tested by Dr. Fleckenstein for HVS genome by DNA-DNA reassociation kinetics and were found to have 47 and 13-14 genomes/cell respectively. Furthermore, HVS-S antigen was detected in the two cell types. Squirrel and owl monkey sera, showing HVS neutralizing antibody, were tested against S antigens from MLC and MEST-HVS cells. Owl monkey sera had the higher titers. These findings, even though preliminary, may parallel the studies reported with EBV antibody from NPC patients. Hybrids prepared with MLC and mouse cells by Drs. Periman and Rabson were unstable. After a few passages, no HVS antigens could be detected in these cells even after stimulation with BudR.

7. An owl monkey was found to shed HVS over a period of one year. Antibodies to various HVS antigens developed during this period. No sign of tumor was detected microscopically or macroscopically, thus suggesting a possible in vivo attenuation of HVS. However, HVS isolated from this owl monkey induced lymphoma and lymphocytic leukemia in cottontop marmosets.

B. Rauscher leukemia virus infected human, nonhuman primate, and rat cells. This Rauscher leukemia virus propagated in nonhuman primate cells (PRLV) was neutralized by rabbit and goat anti-HRV (serum prepared by inoculation of RLV propagated in human cells). PRLV rescued MSV genome from rat kidney cells carrying MSV genome, but this pseudotype virus exhibited one-hit kinetics. Upon inoculation of PRLV in newborn rhesus monkeys and newborn weanling NIH Swiss mice, no disease or symptoms were observed over a period of six months.

C. International Activities

International activities, through Dr. Moloney's office, were expanded.

1. Three visiting scientists, from Germany, France, and India, joined the program as Fogarty Fellows for a period of one year or more.

2. A booklet entitled "Selected Immunological Assays for Oncogenic DNA and RNA Viruses" was prepared for the use of the IARC International Course in Immunovirology of Cancer (given at IARC in Lyon, France from September 30 - October 12, 1974).

3. An NCI-VCP and IARC sponsored International Workshop on Production, Concentration and Purification of Epstein-Barr Virus and Viral Antigens was organized at NCI-FCRC from February 10-12, 1975. Approximately 95 investigators from 13 countries participated in this workshop.

4. The Section supplied reagents and information to various laboratories in the United States and abroad under the WHO/FAO reference laboratory program for comparative virology of simian viruses. We are a collaborating laboratory for the IARC International Reference Program for Oncogenic Herpesviruses.

Significance to Biomedical Research and the Program of the Institute:

A. The sero-epidemiological and immunological data on EBV in AmBL confirm and extend the previous observations. The biochemical and virological studies with EBV and HVS may be of great value in determining cell virus interactions. Subviral and subcellular products may be used in the development of protective vaccines for virally induced neoplasia.

B. The lack of oncogenicity of PRLV both in nonhuman primates and mice suggest a possible attenuation of the RLV. In later studies, PRLV may be useful in vaccine studies. The pseudotype of PRLV and MSV should be tested for oncogenicity, because certain sarcoma viruses have induced tumors in nonhuman primates and other animals.

C. The International Activities in the Virus Cancer Program are significant because they not only build bridges between investigators throughout the world for the exchange of information but allow for the dissemination of standard reagents, training and knowledge about the latest techniques in cancer immunovirology.

Proposed Course:

A. The biochemical, immunological, biological, and chemotherapeutic studies will be continued to aid in understanding the role of human and nonhuman oncogenic viruses in the cause and prevention of cancer.

B. The *in vitro* and *in vivo* studies with PRLV will be continued.

C. The section will continue the coordinating role in the IARC International Reference Center for human tumor associated viral reagents, as well as being an active reference laboratory both for simian viral reagents.

The international activities consisting of the training of foreign scientists, the coordination between the NCI viral oncology and the IARC reference center and supplying and testing of reagents for the reference center and the WHO/FAO simian viruses program will be continued.

Honors and Awards:

Participated as an International Faculty member for the International Course on Immunovirology of Cancer, offered by IARC in Lyon, France in 1974.

Selected by Marquis Who's Who in America Inc. to include biographical sketch in their Fifteenth Edition of Who's Who in the East.

Served as the rapporteur for Session 11 of the Second International Symposium on Oncogenesis and Herpesviruses held in Nuremberg, Germany, from October 14-16, 1974.

Invited by the Director of the Institut fur Klinische Virologie der Universitat Erlangen, Germany to deliver a lecture on Comparative Studies on Epstein-Barr Virus and Herpesvirus saimiri, on September 23, 1974.

Served as the Organizing Chairman of the first IARC-NCI International Workshop on Epstein-Barr Virus which was held at NCI-FCRC, Frederick, Maryland, from February 10-12, 1975.

Appointed from January 1975 as the Secretary to Herpesvirus Advisory Team-1 (HAT-1) of the IARC-International Reference Program for Oncogenic Herpesviruses which is responsible for simian virus reagents.

Publications:

Ablashi, D. V. and Pearson, G.: Herpesvirus saimiri, a nonhuman primate model for Herpesvirus associated neoplasia of man. Proc. Special Virus Cancer Program's International Workshop on "Human tumors associated with Herpesviruses". Cancer Res. 34: 1232-1236, 1974.

Heine, U. and Ablashi, D. V.: Morphological studies of Herpesvirus in primate cell cultures. J. Med. Prim. 3: 18-26, 1974.

Neubauer, R. H., Wallen, W. E., Rabin, H., Pearson, G. R., Ablashi, D. V.: Virological investigation of HVS infected owl monkeys. J. Med. Prim. 3: 27-40, 1974.

Ablashi, D. V., Armstrong, G. R., Easton, J. M., Chopra, H. C., and Adamson, R. H.: Evaluation of effects of cytosine arabinoside on oncogenic Herpesvirus saimiri in owl monkey kidney cells. Biomed. 21: 57-60, 1974.

Twardzik, D. R., Simonds, J., Portugal, F., Armstrong, G. R., and Ablashi, D. V.: DNA polymerase activities in Vero cells infected with Herpesvirus saimiri. Biomed. 20: 1-5, 1974.

Adamson, R. H., Ablashi, D. V., Rabin, H., Cicmanec, J., and Dalgard, D. W.: Chemotherapy of Herpesvirus saimiri-induced lymphoma and leukemia in owl monkey. J. Med. Prim. 3: 68-72, 1974.

Goodheart, C. R., Armstrong, G. R., Ablashi, D. V., and Orr, T. W.: Concentration of oncogenic herpesviruses by methyl alcohol precipitation. Applied Microbiol. 27: 988-990, 1974.

Ablashi, D. V., de Thé, G. B., Easton, J. M., Liabeuf, A., Levine, P. H., and Armstrong, G. R.: Antibodies to Epstein-Barr virus (EBV) antigens in sera of American Burkitt lymphoma patients. Biomed. 20: 288-293, 1974.

Didier, M. L., Ablashi, D. V., Oie, H. K., Armstrong, G. R., Easton, J. M., Chu, E. W., and Rabson, A. S.: Some biological properties of Herpesvirus saimiri from chronically infected monolayer and suspension cultures. In de Thé, G., Epstein, M. A., and zur Hausen, H. (eds.): Oncogenesis and Herpesviruses II. Lyon, France. International Agency for Research on Cancer, WHO, (in press).

Easton, J. M., Ablashi, D. V., de Thé, G. B., Levine, P. H., Twardzik, D. R., Aaslestad, H. G., and Armstrong, G. R.: Association of Epstein-Barr virus (EBV) and American Burkitt's lymphoma (AmBL). In de Thé, G., Epstein, M. A., and zur Hausen, H. (eds.): Oncogenesis and Herpesviruses II. Lyon, France. International Agency for Research on Cancer, WHO, (in press).

Twardzik, D. R., Aaslestad, H. G., Ablashi, D. V., Tureckova, M. I., Gravell, N., and Levine, P. H.: Comparison of DNA polymerase activities from an American Burkitt's lymphoma cell line and EBV producer and non-producer cells. In de Thé, G., Epstein, M. A., and zur Hausen, H. (eds.): Oncogenesis and Herpesviruses II. Lyon, France. International Agency for Research on Cancer, WHO, (in press).

Aaslestad, H. G., Twardzik, D. R., Armstrong, G. R., Zeve, V., and Ablashi, D. V.: Partial purification and biochemical characterization and Herpesvirus saimiri (HVS). In de Thé, G., Epstein, M. A., and zur Hausen, H. (eds.): Oncogenesis and Herpesviruses II. Lyon, France. International Agency for Research on Cancer, WHO, (in press).

Ablashi, D. V. and Easton, J. M.: Approaches to preventive vaccine against Herpesvirus saimiri-induced neoplasia. Symposium on Immunologic Control of Virus-Associated Tumors in Man: Prospects and Problems, Bethesda, Maryland, 1975 (in press).

de Thé, G., Ablashi, D. V., Favre, M. C., Mourali, N., Ellouz, R.: Presence of EBV nuclear antigen in nasopharyngeal carcinoma (NPC) biopsies and their derived cultures. Comparative Leukemia Research, Nagoya, Japan, (in press).

Heine, U., Ablashi, D. V., and Armstrong, G. R.: Morphological studies on Herpesvirus saimiri in subhuman and human cell cultures. In Bellanti, J. A. et al. (ed.): Herpesvirus I. Recent studies. New York, N.Y., MSS Information Corporation, 1974, pp. 183-189.

Ablashi, D. V., Armstrong, G. R., Heine, U., and Manaker, R. A.: Propagation of Herpesvirus saimiri in human cells. In Bellanti, J. A. et al. (ed.): Herpesvirus I. Recent studies. New York, N.Y., MSS Information Corporation, 1974, pp. 219-225.

Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. G., Armstrong, G. R., Rabin, H., Kingsbury, E., and Heine, U.: Induction of lymphoma in owl

monkeys with heated, non-cytopathogenic Herpesvirus saimiri. In Miller, D. et al. (ed.): Oncogenesis and other pathological results of herpesvirus infection: I. New York, N.Y. MSS Information Corporation, 1974, pp. 117-122.

Ablashi, D. V. (ed.): Selected Immunological Assays for Oncogenic DNA and RNA Viruses. Lyon, France. WHO International Agency for Research on Cancer, 1974, 105 pp.

Serial No. Z01-CP-04835-05-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Studies of cell lines infected with MSV in absence of helper virus: Murine sarcoma virus defectiveness

B. Virus search in pediatric solid tumors

C. Development of in vitro human quantitative tissue culture assay systems for detection of potential human RNA tumor viruses

Previous Serial Number: Same

Principal Investigator: Dr. Paul T. Peebles

Other Investigators: Dr. Peter J. Fischinger
Dr. Brenda I. Gerwin

Cooperating Units: Inside NIH
Dr. Alfred Hellman, OB&EC, NCI
Dr. Carl F. T. Mattern, LVD, NIAID
Dr. Edward Scolnick, VLL, NCI

Outside NIH
Dr. George Klein, Karolinska Institutet, Stockholm, Sweden

Man Years:

Total:	3.0
Professional:	1.0
Others:	2.0

Project Description

Objectives:

A. To study rescue phenomena from cell lines infected with murine sarcoma virus (MSV) in the absence of helper viruses and to characterize the expression of the MSV genome in these cells.

B. To search for viruses in pediatric solid tumors.

C. To develop sensitive biological human tissue culture assay systems for the detection of possible human type C viruses.

Methods Employed:

A. Evidence for sarcoma and leukemia virus production in cell cultures were measured by known tissue culture assays, biochemical assays, immunological tests, and electron microscopy.

B. Recently developed human tissue culture detection systems have been utilized in an attempt to find evidence for a viral etiology in pediatric tumors.

C. Human tissue culture cell lines are being investigated for their potential use as detection systems for human oncorna viruses using techniques previously employed for investigation of MSV and MuLV.

Major Findings:

A. To study the expression of the defective "S+L-" type MSV genome in human cells, MSV rescued from cloned sarcoma-positive leukemia-negative mouse cells was used to obtain a single cell clone, termed S+L- HuACl_1, from a "single-hit" terminal focus in human AV-3 amnion cells. Second generation MSV, rescued from S+L- HuACl_1 cells by superinfection with RD-114 virus, was employed for generating three clones of S+L- cells derived from three separate MSV foci in L-132 human lung cells. S+L- human amnion and lung cell clones demonstrated certain characteristics of S+L- mouse cells; all four S+L- human cell clones were transformed, expressed the genetically stable murine leukemia virus group-specific (gs-1) antigen marker, and contained cryptic MSV rescuable by superinfection with compatible helper-type viruses which conferred host range properties on the rescued MSV pseudotypes. S+L- human cells differ from S+L- mouse cells in that S+L- human cells do not release detectable type C virus-like particles. S+L- human cells also lack detectable quantities of the viral core protein reverse transcriptase. S+L- human cells, though similar to nonproducer Kirsten-MSV transformed mouse cells, fail to release detectable MSV after iododeoxyuridine induction. Noninfectious type C virus particle production may not be an expression of the S+L- MSV genome. However, if the potential of defective particle production is encoded in this particular "S+L-" MSV genome, then human cells may have control mechanisms for suppressing this function or they may lack a factor present in mouse cells which permits defective particle production. Also, the "S+L-" type MSV might have lost this function after passage through feline and human cells.

The nature of murine sarcoma virus (MSV) defectiveness was studied by examining MSV rescued by RD-114 virus from MSV nonproductively transformed human cells. The rescued MSV pseudotype, which displayed the viral envelope-associated properties of RD-114 virus, was in approximately 100-fold biologically detectable excess over attending RD-114 helper virus. The

biologically determined virus ratio correlated with the ratio of MSV to RD-114 virus determined by viral nucleic acid hybridization. With antisera which distinguish the DNA polymerase of murine type C virus from that of RD-114 virus, it was demonstrated that at least 70% of the RNA dependent DNA polymerase (RDDP) of the 99% MSV stock is that of RD-114 virus while no murine virus polymerase is detectable. The detection of only helper virus RDDP in the MSV excess stock along with the known absence of detectable RDDP in all heterologous host S+L- human, dog, and mink cells indicate that the MSV genome lacks information necessary for polymerase and actually acquires its core protein RDDP from rescuing helper virus.

Sarcoma virus-positive leukemia virus-negative (S+L-) mouse cells transformed by defective but resuable murine sarcoma virus (MSV) release noninfectious type C virions with a quantitative deficiency of viral-type RNA-dependent DNA polymerase (RDDP). Human S+L- cells containing the same genome fail to express any detectable viral-type reverse transcriptase. To study this difference in MSV expression, second generation MSV from the S+L- human cells is recloned in dog, mink, and back into mouse cells. Heterologous host dog and mink S+L- cell clones, like human S+L- cell clones, fail to release viral-type reverse transcriptase into culture supernatants. All second generation homologous mouse S+L- cell clones again release viral reverse transcriptase in supernatants indicating that the MSV genome has not been altered in this function. Using $(dT)_{12-18}$ cellulose and phospho-cellulose chromatography of cellular polymerase preparations, no intracellular buildup of unreleased murine reverse transcriptase is detected in the S+L- clones.

The MSV genome seems to be at least in part if not totally defective in the information for viral RDDP. The minimal amount of murine viral polymerase consistently present in noninfectious virions released only from homologous host S+L- mouse cell clones is apparently an expression of a recently isolated helper virus endogenous to murine cells. Also, the stability of the MSV genome in heterologous host cells and its failure to demonstrate any evidence to date of recombination with nonmurine replicating (r+) but nontransforming (t-) helper viruses suggests that replication defective (r-) but transforming (t+) MSV is not generated during replication by simple genetic reassortment or substitution of subunit t for r but has evolved by intramolecular recombination at some distant time with murine leukemia virus (MuLV). MSV cloned in heterologous host cells lacking endogenous murine type C viruses and permitting MSV rescue by helper viruses nonhomologous with MuLV should facilitate molecular studies of MSV and MuLV.

B. The experiments screening human neoplasias for candidate oncorna viruses using normal and S+L- human cells remain negative. Primary cultures of tumor cell strians, and cell lines were examined. These consisted of lymphomas, leukemias, carcinomas and other assorted representative pediatric tumors.

The tests employed were co-cultivation, RD-114 superinfection, Sendai virus fusion, mixed lymphocyte, and heterologous host cells cocultivation. Also the neural-brain tumors were found to be negative for SV-40 T antigen.

C. A rapid focus induction assay has been developed for replicating (r+) but nontransforming (t-) type C viruses including xenotropic murine leukemia virus (X-MuLV), feline leukemia virus C (FeLV-C), and feline-primate viruses RD-114/CCC/M-7. Mink cells infected with r-t+ defective murine sarcoma virus (MSV) while phenotypically flat, become transformed and form foci with "1-hit" kinetics when superinfected with several r+t- type C viruses. The assay may be utilized for rapid and direct neutralization of the compatible r+t- viruses without resorting to sarcoma virus pseudotype production and may also be employed for cloning of compatible r+t- mammalian viruses.

Significance to Biomedical Research and the Program of the Institute:

A. The exploration of murine oncogenic RNA viruses, their defectiveness, and the variance between their capacity to replicate and their capacity to transform has great implications in that their existence makes probable the viral etiology for human cancer.

B. The tumors from pediatric patients are of particular interest for a tumor virologist because of the possibility of vertical transmission of oncogenic viruses. Also clinically, outside of automobile and ingestion accidents, cancer is responsible for most of the deaths in children in the United States.

C. The interrelationship between a sarcoma virus and human cells has been demonstrated to be identical to that in known animal models. "Defective" MSV is able to infect and transform human amnion and lung cells but is unable to replicate infectious progeny focus-forming virus in the absence of replicating helper virus. This relationship may be operating in some human tumors and the inability to find infectious virus may be due to the presence of defective transforming sarcoma genomes in the absence of detectable replicating leukemia viruses.

Proposed Course:

A. We are attempting to further characterize the phenotypic expression of MSV in the heterologous host S+L- cells in comparison to mouse S+L- cells.

B. We are continuing to attempt to demonstrate a viral etiology in pediatric solid tumors.

C. Certain aspects of the tissue culture system using human cell lines susceptible to MSV transformation are being studied in order to improve the changes for detection of human sarcoma viruses. Rapid tissue culture assays are being developed using S+L- cells for possible detection of replicating nontransforming human type C viruses.

Honors and Awards:

A faculty member for the course on "Principles of Biohazards and Injury Control in the Biomedical Laboratory," University of Minnesota School of Public Health. His lecture was entitled "Personal Practices and Tissue Culture Techniques in Tumor Virus Research."

Presented a talk on "Murine Sarcoma Virus Defectiveness" to the Institute of Allergy and Infectious Diseases, NIH, March 20, 1974.

On the Academic Staff of the George Washington University School of Medicine in the status of Assistant Clinical Professor of Child Health and Development for consultative-teaching services to the Children's Hospital National Medical Center, Washington, D. C.

Publications:

Peebles, P. T., Fischinger, P. J., and Newman, A. J.: Human amnion and lung tissue culture systems for possible detection and study of human RNA tumor viruses. In Ito, Y. and Dutcher, R. (Eds.): Comparative Leukemia Research, 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 363-371.

Fischinger, P. J., Nomura, S., and Peebles, P. T.: Control of reversion of MSV transformed cells. In Ito, Y. and Dutcher, R. (Eds.): Comparative Leukemia Research 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 475-481.

Papageorge, A. G., Peebles, P. T., Gerwin, B. I., Fischinger, P. J., and Mattern, C. F. T.: Expression of the defective "S+L-" type murine sarcoma virus genome in human amnion and lung cells. J. Natl. Cancer Inst., 52: 1727-1737, 1974.

Hellman, A., Peebles, P. T., Strickland, J. E., Fowler, A. K., Kalter, S. S., Oroszlan, S., and Gilden, R. V.: Baboon virus isolate M-7 with properties similar to feline virus RD-114. J. Virol. 14: 133-138, 1974.

Serial No. Z01-CP-04836-01-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause
   and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Project
July 1, 1974 through June 30, 1975

Project Title: Detection of mammalian type C viruses and their proteins in normal and malignant tissues

Previous Serial Number: None

Principal Investigator: Dr. Joseph E. DeLarco

Other Investigators: Dr. R.E. Benveniste
Dr. R. Callahan
Dr. C.J. Sherr
Dr. G.J. Todaro

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

Experiments will be carried out to isolate and characterize mammalian type C viruses and to define the cellular control mechanisms regulating the expression of these viruses and their proteins.

Methods Employed:

Cell lines from various mammalian species will be used to isolate unknown viruses and to characterize their host range. New viral isolates will be further characterized by electron microscopy, viral reverse transcriptase assays, radioimmunoassays for the intact virus and the viral proteins, and by DNA:DNA and DNA:RNA hybridization experiments.

Major Findings:

There are no significant new findings at this time.

Significance to Biomedical Research and the Program of the Institute:

These studies will be carried out in order to further characterize mammalian type C viruses and provide knowledge concerning the control mechanisms regulating their expression and the expression of their proteins. The relationship of type C viruses to the etiology of human neoplasia will be actively pursued.

Proposed Course:

An attempt will be made to isolate type C viruses from normal human tissue or tumor specimens and examine them as possible candidates for the causative agent in human cancer. Studies will be carried out to determine if antibodies to type C viruses can be located in the serum of normal subjects or patients with neoplasia. Other experiments will be performed to further characterize the cellular regulation of known mammalian endogenous type C viruses.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04837-01-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immune recognition of nascent autochthonous tumors

Previous Serial Number: None

Principal Investigator: Dr. William J. Martin

Other Investigators: Dr. Tadao Aoki

Cooperating Units: Inside NIH
Mrs. Sue Ellen Martin, LBG, NHLI

Outside NIH
None

Man Years:

Total:	1.50
Professional:	1.00
Other:	0.50

Project Description

Objectives:

Detection and characterization of tumor related cell surface antigens by which the immune system detects and eliminates nascent autochthonous tumors.

Methods Employed:

In vitro cultivation of tumor cells of both mesodermal and non-mesodermal origin. Antigenic analysis of the tumor cell lines using both immune and naturally occurring antibodies in the complement dependent cytotoxicity assay.

Major Findings:

Normal mouse sera were shown to contain a wide variety of naturally occurring antibody (NOA) reactive with both recently derived and long term

transplantable tumor cell lines. The occurrence of NOA in sera of congenitally athymic (nude) mice indicated that the production of these antibodies was thymus independent. Absorption studies revealed that the various tumor cell lines expressed both individually distinct and shared antigens. An antigen recognized by NOA on a murine neuroblastoma cell line was found to be present on normal brain tissue of species as diverse as chicken and man. Many of the tumor antigens recognized by NOA could not, however, be detected on normal tissues and appeared to be tumor specific. A strain of mice was identified which had a genetically determined defect in the production of tumor reactive NOA. These mice were found not to have an unusually high incidence of spontaneous tumors.

Significance to Biomedical Research and the Program of the Institute:

While it has generally been assumed that the immune system plays an important role in the detection and elimination of nascent autochthonous tumors, little direct evidence in support of this hypothesis has been forthcoming. Indeed, the recent demonstration that congenitally athymic (nude) mice do not have an increased incidence of spontaneous tumors has seriously challenged the importance generally attached to T cell immunity in immune surveillance. The finding of naturally occurring tumor reactive antibodies in sera of both normal and congenitally athymic mice raised the possibility that humoral rather than T cell immunity might be responsible for immune surveillance against tumors. Since at least some NOA recognized normal tissue antigens and since mice genetically defective in production of NOA do not have an increased tumor incidence, it is unlikely that NOA act in a quantitative manner in the destruction of nascent tumors. The biological function of NOA remains an open question. Nevertheless, the study of NOA provides a sensitive method with which to detect both tumor-associated and normal tissue antigens and may help in the identification and immmunological control of malignant disease.

Proposed Course:

This project will be continued outside the Viral Oncology Area.

Honors and Awards:

None

Publications:

Martin, W.J.: Immune surveillance directed against derepressed cellular and viral alloantigens. Cell. Immunol. 15: 1-10, 1975.

Martin, S.E. and Martin, W.J.: Anti-tumor antibodies in normal mouse sera. Int. J. Cancer, in press.

Martin, S.E. and Martin, W.J.: Interspecies brain antigen detected by naturally occurring mouse anti-brain autoantibody. Proc. Natl. Acad. Sci. USA, in press.

Serial No. Z01-CP-04838-01-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause and Prevention
2. Viral Biochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: An investigation of the mechanism(s) of host range restriction of exogenous and endogenous leukemia viruses

Previous Serial Number: None

Principal Investigator: Dr. Martine Canivet

Other Investigators: Dr. Robert H. Bassin
Dr. Brenda I. Gerwin

Cooperating Units: Inside NIH
Staff, VLLB, NCI

Outside NIH
Dr. Alan Rein, Litton Bionetics, Inc., Bethesda, Md.

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description

Objectives:

To study the nature of the host range restriction in N-type cells infected with B-tropic virus, and in B-type cells infected with N-tropic virus.

Methods Employed:

Routine methods for in vitro propagation of cells and virus and selection of cloned lines of cells by soft agar and microplating techniques were employed. Detection of virus was determined by presence of reverse transcriptase. Quantitation of virus infection employed the S+L- and XC assay techniques for leukemia virus infectivity. Quantitation of producer cells involved infectious center assays on N- and B-type cell lines.

Major Findings:

These experiments have recently been initiated. Initial results indicate that B-tropic virus is able to replicate in N-type cell lines, but that the amount of infectious virus produced is reduced by about 100-fold.

Significance to Biomedical Research and the Program of the Institute:

These experiments will provide information concerning the nature of cellular restriction of N- and B-tropic MuLV's.

Proposed Course:

A systematic biochemical and virological analysis of clones derived from N-type cells infected with B-tropic virus will be initiated. The percentage of producing clones and the host range properties of the virus produced will be determined. Nonproducing clones will also be examined for evidence of virus expression.

Honors and Awards:

Dr. Canivet is a Visiting Associate of the National Cancer Institute.

Publications:

None

Serial No. Z01-CP-04839-02-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Study of the host range specificity of MSV rescued by N- and B-tropic MuLV.

B. Study of the behavior of N- and B-type cells coinfected with N- and B-tropic viruses.

Previous Serial Number: Same

Principal Investigator: Dr. Graciela Duran-Troise

Other Investigators: Dr. Robert H. Bassin

Cooperating Units: Inside NIH
Staff, VLL, NCI

Outside NIH
None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

A. To determine whether the host range specificity of N- and B-tropic MSV pseudotypes is independent of the MuLV host range restriction mechanism.

B. To elucidate the behavior of N-type cells and B-type cells when infected with both N- and B-tropic virus simultaneously.

Methods Employed:

Routine tissue culture methods are employed. The MSV pseudotypes utilized in these experiments are rescued by superinfection of mouse cell lines transformed with the Moloney, Kirsten and Harvey isolates of MSV.

Transformation by MSV is quantitated in C3H, BALB/3T3 and 3T3FL cells by focus formation in the presence and absence of optimal amounts of MuLV, "helper" virus.

Major Findings:

A. The focus formation assays in N-type cells (NIH/3T3) and B-type cells (BALB/3T3) using S+L-, H-MSV, Ki-MSV, and MSV-85 pseudotypes showed a similar behavior. It indicates that the N- or B-tropism of the MSV is specified by the type of MuLV used for the rescue of the MSV. Cell transformation is restricted as well as MSV replication.

With the H-MSV, S+L- MSV and MSV-85 pseudotypes it seems that focus formation in B-type cells (BALB/3T3) is more restricted. On the contrary, with Ki-MSV pseudotypes it seems that the N-type cells are more restrictive than B-type cells.

B. These experiments are in progress; no conclusions have been reached as yet.

Significance to Biomedical Research and the Program of the Institute:

A. These experiments will provide information about the mechanism whereby host cells are able to inhibit the replication of certain types of RNA tumor viruses while permitting the replication of other closely related types. They will also provide information concerning the conditions under which host range restrictions of the cells can be altered and how viruses can overcome cellular control mechanisms in certain cases.

B. Evidence concerning recombination between N- and B-tropic viruses is important to an understanding of the replicative cycles of RNA tumor virus.

Proposed Course:

A. Further examination of N- and B-tropic MSV pseudotypes from clones of C3H cells transformed by Ki-MSV(B) and 3T3 cells transformed by S+L- MSV(N) is proposed in order to know by which mechanism(s) in a nonpermissive system some cells are permissive to virus replication.

B. Focus forming assays on N-type cells and B-type cells systems with N- and B-tropic S+L- MSV pseudotypes and N, B, and NB helper virus will be performed in order to determine the nature of virus-virus interactions in these systems after dual infection.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04840-05-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Non-human primate surgery - development of special techniques and procedures in addition to routine surgery

Previous Serial Number: Same

Principal Investigator: Dr. Sergio A. Leiseca

Other Investigators: Dr. Michael R. Mardiney, Jr.
Dr. Michael A. Chirigos

Cooperating Units: Inside NIH
None

Outside NIH
Litton Bionetics Research Laboratories
Kensington, Maryland

Man Years:

Total:	1.00
Professional:	1.00
Other:	0.00

Project Description

Objectives:

1. To provide the National Cancer Institute, and other cooperating institutes, with embryonic and fetal material from the primate colony maintained at Litton Bionetics Research Laboratories (BRL).

2. To provide surgical procedures required to maintain and improve the health of non-human primates.

Methods Employed:

1. Cesarean sections technique performed on primates (primarily Macaca mulatta).

2. Developed and applied procedures for cannulation of thoracic lymph ducts.

3. Hysterotomy and biopsy of fetal skin and muscle.

4. Laparatomy in non-human primates.

5. Many operative procedures are done to repair various clinical conditions.

6. Biopsy performed in primates.

Major Findings:

1. C-section techniques are also used for the following: to obtain fetal material for large amounts of primary cell cultures used in NCI; to provide most of the infants for the V.C.P. virus inoculation program at the Litton Bionetics Research Laboratories project; to study the effect of carcinogenic materials on fetuses inoculated in utero; to provide through amnioncestesis recovery of drug in the amniotic fluids for the determination of possible inducement of anomalies or tumors, and to salvage all placentae for protein studies in relation to malignancy and birth control.

2. Cannulation of thoracic lymph ducts is used in rhesus monkeys for the continuous collection of lymph in the study of the malignant tumors.

3. The C-section technique is used to present the fetuses and neonatal animals for immunosuppressive studies and for inoculation of leukemia or other cancerous materials as well as some viruses. Biopsies of fetuses are used for tissue cultures for later infection with cancerous materials and reinjection into the donor.

4. Laparotomy technique is used in the animals for studies of inoculation of leukemia or cancerous materials.

5. Surgical procedures are routinely performed to repair injuries resulting from accidents that occur in the primate breeding colony. Some of these procedures are: (a) suture and debridement of various wounds, and lacerations of head, body, extremities, tongue, tail; (b) drainage of abscess; (c) amputations of digits traumatized and tail; (d) open reduction of fractures; (e) herniorrhaphy-umbilical, vential, or inguinal; (f) closure of wounds; (g) C-section to obtain fetuses in breech presentation, bleeding due to premature placental separations, toxemia, protracted labor, prolonged or overdue pregnancy, dead fetus, etc.; (h) prolapse of rectus; (i) prolapse of vagina and cervix; (j) D & C to remove decidua following abortion or delivery; (k) tumor biopsy; and (l) laparotomy for ovarian cyst, ruptured uterus, splenomegaly, etc.

6. Biopsy techniques are also used to obtain material and study the effects of carcinogenesis. We performed biopsies of different organs, such as: liver, spleen, lung and mammary glands.

Significance to Biomedical Research and the Program of the Institute:

Non-human primates have proven very useful in research on human viral diseases. They are man's closest phylogenetic relative and are the most desirable model system in research projects which cannot utilize humans directly. Much of the work in primate cancer research is dependent on the highest quality surgical procedures for the provision of sterile fetal tissue and healthy young free of pathogenic organisms. Surgical intervention for lymph and other tissues requires great professional skill for maximum animal health and comfort in addition to experimental validity.

Proposed Course:

Continue to provide the best possible surgical procedures for primate cancer research.

Honors and Awards:

None

Publications:

Martin, D.P. and Leiseca, S.A.: Effect of oral contraceptives on the mammary glands of rhesus monkeys. Animal Research Journal, In press.

Serial No. Z01-CP-04841-01-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The state of viral genome in revertant cells

Previous Serial Number: None

Principal Investigator: Dr. Arthur E. Frankel

Other Investigators: Dr. Peter J. Fischinger
Dr. Daniel K. Haapala

Cooperating Units: Inside NIH
None

Outside NIH
None

Man Years:

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

To determine the nature of cellular control of the MSV genome in revertant cells of several species.

Methods Employed:

RNA-DNA and DNA-DNA hybridization using hydroxyapatite and S1 nuclease and endogenous reverse transcriptase probe. Virus is assayed by leukemia virus helper assay, focus induction in S+L- cells.

Major Findings:

A. In long term culture productively infected S+L- cells can maintain a sarcoma:leukemia ratio of 1.

B. Using actinomycin D an oncornavirus endogenous reverse transcriptase product can be made with less than 10% double stranded DNA and good (70%) hybridization to homologous RNA.

C. Cat cells infected with MSV contained MSV RNA. Revertants from these transformed cells did not contain MSV RNA. The viral DNA content is presently being investigated.

Significance to Biomedical Research and the Program of the Institute:

A. The mechanism of control of the MSV genome in revertant cells may be applicable to control of human cancer cells.

B. The development of techniques to dissect the expression of tumor information will be applicable for use with human tumor viruses when they are found.

Proposed Course:

A. To screen different transformed and revertant cells for viral RNA and DNA content. Hopefully, to find part or all of the viral DNA in a non-transcribed state in some cells.

B. The study of the control mechanisms at work in A. by in vitro transcription.

C. To evaluate different nonpermissively transformed cells expressing different viral functions and hence to see if certain viral RNA sequences can be isolated which contain specific properties on translation (i.e., transformation).

D. To evaluate the state of the MSV genome in these cells as to whether the DNA is integrated or in the plasmid state.

Honors and Awards:

None

Publications:

None

Serial No. Z01-CP-04842-04-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer
Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The biochemistry and biophysics of leukemia and sarcoma viruses

Previous Serial Number: Same

Principal Investigator: Dr. Leo A. Phillips

Other Investigators: Dr. Peter J. Fischinger
Dr. Arthur E. Frankel
Dr. Daniel K. Haapala
Dr. Shigeko Nomura
Dr. Roy H. L. Pang
Dr. Paul T. Peebles

Cooperating Units: Inside NIH
Dr. Robert J. Crouch, LMG, NICHHD
Dr. Adi F. Gazdar, VLL, NCI
Dr. Vincent W. Hollis, VLL, NCI

Outside NIH
None

Man Years:

Total:	3.0
Professional:	2.0
Others:	1.0

Project Description

Objectives:

These studies are concerned with the biochemical and biophysical properties of nucleic acids and polypeptides from RNA-tumor viruses. Our objectives are as follows:

1. The characterization of the nucleic acids and the structural polypeptides of purified virions released from cells which have been transformed by sarcoma viruses.

2. The isolation and characterization of the viral specific nucleic acid and polypeptides of cells infected with leukemia and sarcoma viruses.

3. The determination of which of the viral specific polypeptides is the reverse transcriptase of the virions.

4. The determination of what makes a sarcoma virus either competent or defective.

5. The determination of the state of the sarcoma viral DNA in S+L- cells which are not releasing particles; a determination of whether the DNA exists as a plasmid, an episome, or whether it is integrated into the cellular DNA.

6. The determination of what makes one cell line permissive to oncogenic virus infection and another cell line nonpermissive.

7. The examination of human cancer materials (neoplasias) for viral specific nucleic acids.

8. Further biochemical and biophysical characterization of the mammalian sarcoma virus genome in the absence of replicating leukemia virus and in leukemia-sarcoma virus systems with an excess of the sarcoma genome.

9. The determination of the capacity of the covalently linked 3'-terminal poly·(A) sequence to act as primer for reverse transcriptase.

10. Partial sequences analysis of genomic and subunit viral RNA.

11. The determination of the number of viral RNA subunits present in the viral genome of leukemia virus and the leukemia-sarcoma virus complex.

12. The determination as to whether the viral RNA subunits are the same (homoploidy) or different (heteroploidy).

13. The determination as to whether RNase H and reverse transcriptase are one enzyme with two separate mechanisms of action or whether they are two separate enzymes with similar physical characteristics.

14. The determination of the role of poly (A), poly (C), and poly (G) sequences in genomic and subunit viral RNA.

15. A study of the effect of conformational changes of both genomic and subunit viral RNA on transcription and translation.

16. Studies to determine the degree of stabilization of viral RNA by poly (A) after transcription and translation.

17. Studies of possible transformation by (a) RNA-DNA hybrids and (b) genomic viral RNA before and after incubation with reverse transcriptase.

Methods Employed:

The focus assay for sarcoma viruses and the helper assay for leukemia viruses are employed for the quantitation of the viruses. [^{3}H]-uridine incorporation in particles with a density of 1.16 g/cm^3, RNA-dependent DNA polymerase assays on supernatant fluids and electron microscopy are utilized to determine if virus is released from transformed cells. For characterization of the viral nucleic acids and polypeptides, standard biochemical and biophysical techniques (analytical and preparative ultracentrifugation, column chromatography, RNA·DNA hybridization, isotopic labeling, polyacrylamide gel electrophoresis, peptide fingerprinting, electrofocusing, in vitro protein synthesis, enzymology, etc.) are being employed.

Major Findings:

The RNA from noninfectious virions produced by two established clonal lines of sarcoma-positive leukemia-negative (S+L-) transformed 3T3 cells has been characterized, RNA from virions or nucleoids of S+L-(C243) cells consisted of 3 to 4 sizes: ±44S (6%), 28S (17%), 18S (38%) and less than 18S (30%). The 28S virion RNA was found to contain virus specific information demonstrable by RNA·DNA hybridization with a DNA probe derived from the murine sarcoma-leukemia virus reverse transcriptase product. The RNA from virions or nucleoids of S+L-(D56) cells consisted of 5 sizes: 80S (6%), 68S (8%), 56S (5%), 28S (28%), and <28S (53%). Comparative parallel studies with rRNA from 60S subunits of transformed and nontransformed 3T3 cells, determined that the 28S S+L- virion RNA was most probably the monomeric genome because it contained virus specific information and poly·(A) sequences at the 3'-terminus. The 56S, 68S, and 80S RNA are most probably tetrameric, hexameric, and octomeric genome aggregates.

Location of polyriboadenylate sequences in the RNA of mammalian leukemia and sarcoma viruses was determined by submitting the 56-64S genomic and 28-35S subunit viral RNAs, and the 4-5S polyriboadenylate sequences excised from these viral RNAs to either hydrolysis with purified exoribonuclease specific for 3'-OH termini or phosphorolysis from the 3'-OH termini with polynucleotide phosphorylase. The kinetics of these enzymatic reactions, as determined by hybridization of the residual [2,8-^{3}H] adenosine labeled RNA to polyuridylate GFC filters, indicated that the polyriboadenylate sequences are at the 3'-termini of ribonucleic acid from RNA-tumor viruses. Possible functions of the 3'-terminal polyriboadenylate sequences are considered and a genomic model is proposed with the covalently linked 3'-terminal polyriboadenylate sequences functioning as primer for reverse transcriptase.

The nucleotide sequences in genomic viral RNA (50±6S vRNA) from purified virions isolated from sarcoma-positive and helper-positive hamster cells (S+H+) have been examined by polynucleotide agarose affinity chromatography. The virus was pulse-labeled with either [2,8-^{3}H]-adenosine or $H_3{}^{32}PO_4$ for 3 hrs and 50±6S vRNA was extracted. Ninety percent of [2,8-^{3}H-A]-labeled

50±6S vRNA bound to poly (U) agarose column, indicating that there is at least one copy of poly (A) sequence per 50±6S vRNA molecule. In addition 53% of heat-denatured genomic vRNA and 10% of RNase T_1-treated vRNA bound to poly (U) agarose column. Degradation of 50±6S vRNA was not observed after passing vRNA through poly (U) agarose column. After heat denaturation of 50±6S vRNA, 25-28S vRNA species, with or without poly (A) sequences were detected in 15 to 30% isokinetic sucrose gradient. There is no detectable amount of poly (U) tracts observed in either native or heat-denatured 50±6S vRNA after passing vRNA through poly (A) agarose column. However, native or heat-denatured 50±6S vRNA bound to poly (C) and poly (G) agarose columns, although lesser poly (G) tracts than poly (C) tracts were observed. In addition, heat-denatured 50±6S vRNA has a greater affinity for poly (G) agarose column than native 50±6S vRNA. The poly (C) and poly (G) tracts detected may play a role in the secondary structure of oncorna viral RNA.

Studies were initiated for the selective isolation of virus specific RNA extracted from cytoplasmic lysates of [2,8-^{3}H] adenosine-labeled 3T3 mouse cells chronically infected with a Moloney leukemia virus isolate (MLV-IC) and sarcoma-positive helper-positive (S+H+) hamster cells. The cytoplasmic RNA was fractionated by poly (U)-agarose affinity chromatography into poly (A) negative (∿95%) and poly (A) positive (∿5%) RNA. RNA molecules containing poly (A) sequences were separated by rate zonal sedimentation analysis.

Isokinetic sucrose gradient profiles of RNA obtained from these cytoplasmic lysates before poly (U)-agarose fractionation appear to consist of predominantly 28S, 18S, 4-5S ribosomal RNA peaks, and no 50-70S genomic viral RNA. In contrast, sedimentation profiles of the poly (U)-agarose fractionated poly (A) positive cytoplasmic RNA from these virus producing cells consisted of 30±6S and 13±6S RNA peaks, no prominent 50-70S genomic viral RNA. Poly (U)-agarose fractionated cytoplasmic RNA from noninfected 3T3 cells contained only messenger RNA molecules because ribosomal RNA does not contain poly (A) sequences. RNA-DNA hybridization studies are in progress to determine which of the poly (A) sequences containing cytoplasmic RNA molecules are of viral and cellular origin.

Significance to Biomedical Research and the Program of the Institute:

These biochemical and biophysical studies of nucleic acids and polypeptides from RNA-tumor viruses contain three avenues for obtaining pertinent knowledge for the possible cure of cancer: (1) studies which are being conducted on the viral nucleic acids and structural polypeptides could possibly determine if their mode of replication is by means of transcriptional-translational complexes, and if so, it may be possible to effect a cure for cancer by specifically blocking the replication of these RNA tumor viruses either at the transcriptional or translational level;

(2) studies which are being done to determine the disposition of the viral genome in sarcoma transformed, nonproducer cells could possibly enable the excision of the viral genome without affecting the cellular genome; (3) studies which are being conducted to ascertain the property which makes one cell line permissive, while another cell line is nonpermissive, to infection by RNA tumor viruses may be valuable in effecting a cure for cancer.

Proposed Course:

These critical biochemical and biophysical studies of nucleic acids and polypeptides from RNA tumor viruses will be continued in order of their complexity.

Honors and Awards:

Selected for 1974 Who's Who in America - East Volume.

Invited to give the following seminars:

1. "Nucleotide Sequences in the RNA of Mammalian Oncornaviruses", Atlanta, Georgia, November 22, 1974.

2. "Nucleotide Sequences in RNA of Oncornaviruses", Howard University, Washington, D. C., December 18, 1974.

3. "Nucleotide Sequences in the RNA of Mammalian Leukemia and Sarcoma Viruses", Louisiana State University, Baton Rouge, Louisiana, Feb. 7, 1975.

4. "Nucleotide Sequences in the RNA of Mammalian RNA-tumor Viruses", NIH, Bldg 10, Rm 3B08, Bethesda, Md., March 19, 1975.

Selected to be Chairman of an RNA-tumor virus session at the American Society for Microbiology meeting in New York, New York, April 27-May 1, 1975.

Publications:

Phillips, L. A., and Bussell, R. H.: The nucleic acid of canine distemper virus: Effects of 6-azauridine and actinomycin D on viral replication, and the incorporation of uridine into virions. Am. J. Vet. Res. 35: 821-824, 1974.

Phillips, L. A., Park, J. J., and Hollis, V. W., Jr.: Polyriboadenylate sequences at 3'-termini of ribonucleic acid obtained from mammalian leukemia and sarcoma viruses. Proc. Natl. Acad. Sci. USA 71: 4366-4370, 1974.

Pang, R. H. L., Phillips, L. A., and Park, J. J.: Nucleotide sequences in the RNA of mammalian oncornaviruses. Bacteriol. Proc., in press.

Phillips, L. A., Park, J. J., and Pank, R. H. L.: Selective isolation of virus specific RNA from mammalian leukemogenic and sarcomogenic cells. Bacteriol. Proc., in press.

Serial No. Z01-CP-04843-01-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause
   and Prevention
2. Primate Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on oncogenic herpesviruses of man and subhuman primates.

Previous Serial Number: None

Principal Investigator: Dr. Josef Luetzeler

Other Investigators: Dr. D. V. Ablashi
Dr. J. M. Easton

Cooperating Units: Inside NIH
Dr. Paul Levine, VLLB, NCI
Dr. Jean Guegan, NCI

Outside NIH
Dr. Peter Ebbesen, Institute of Medical Microbiology, University of Copenhagen, Copenhagen, Denmark
Dr. Daniel Twardzik, Frederick Cancer Research Center, Frederick, Maryland
Dr. Halvor Aaslestad, Frederick Cancer Research Center, Frederick, Maryland
Dr. Gerhard Krueger, Pathology Department of the University of Cologne, Cologne, West Germany

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

A. To study Epstein-Barr virus (EBV) serologic specificities in sera from nasopharyngeal carcinoma (NPC) patients from Hong Kong, Tunisia, the U.S.A., and other European countries.

B. To study the effect of HPA-23 on the expression of EBV viral capsid antigen (VCA) in P_3HR1 cells showing no VCA production.

C. To study two strains of Herpesvirus ateles (HVA) with induction and comparison of early and late membrane antigen (MA) and confirmation of antigenic differences between the strains.

Methods Employed:

A. In the studies of NPC sera standard methods were used. VCA was tested in P_3HR1 cells; EA was assayed by superinfecting Raji cells with EBV; EBNA was assayed in Nunn cells; whereas MA was assayed in Ly-28 cells, using indirect immunofluorescence techniques. A standard microtiter complement-fixation (CF) test was employed for the detection of soluble CF antibodies to EBV.

B. In the studies on the effect of HPA-23, P_3HR1 cells were used, which had stopped producing EBV-VCA. Cells were treated with 20 µg/ml of HPA-23 (toxic level 30 µg/ml) for 5 days at 36°C.

C. In the studies on two strains of Herpesvirus ateles (HVA-810, HVA-73), owl monkey kidney (OMK) cells and marmoset monkey kidney (MMK) cells were used. For determination of MA in HVA-infected OMK and MMK cells, simian sera were employed, and for determination of early and late MA, a DNA synthesis inhibitor (cytosine arabinoside) was used.

Major Findings:

A. The sera from Hong Kong and Tunisia had higher geometric mean antibody titers (GMT) than the sera from American patients and the controls to all IF antigens of EBV. VCA, EA, MA, and CF titers correlated very well with each other; however, EBNA titers didn't show a significant correlation. MA titers were always lower than VCA and EA. The GMT of the sera from Hong Kong and Tunisia for VCA, EA, and MA are 925, 200, and 66 respectively.

B. HPA-23 stimulated the growth of B-lymphocytes and enhanced the VCA and EA expression in P_3HR1 cells to 15%, while untreated P_3HR1 cells showed no VCA. Upon stimulation with IudR, P_3HR1 cells showed only 4-5% VCA and EA. No induction of EA in Raji cells was observed upon treatment with HPA-23.

C. In studies with HVA-73 and HVA-810 a very slow-progressing cytopathic effect (CPE) was found. The $TCID_{50}$ for HVA-73 was about 10^5/ml. In the case of HVA-810, there was a persistent problem in achieving a high infectious titer. The $HVA_3$810 grew very poorly in OMK cells, and the titers were never greater than 10^3 $TCID_{50}$/ml.

Significance to Biomedical Research and the Program of the Institute:

A. The serologic data shows a strong relationship between EBV and NPC, with high antibody titers in Chinese and Tunisian NPC patients, whereas the sera from American NPC patients had low titers to various EBV-related antigens. Due to very few samples from American patients, no further conclusions can be drawn; however, it is evident that EBV is related in some way to NPC of Chinese and Tunisian origin.

B. The enhancement of production of EBV antigens may be ultimately useful in making these available in larger quantities for related biomedical research.

C. The studies on HVA will confirm the antigenic difference between strain 73 and 810 by virus neutralization and MA induction. Because HVA-73 is a transforming virus of lymphocytes, the membrane components of HVA-73 and HVA-810 may very well differ in their properties. The membranes of HVA-810 may be quite useful in vaccine studies as a primate model for human tumors with a suspected virus etiology.

Proposed Course:

A. NPC sera will continue to be studied for the activities against various EBV antigenic specificities and for epidemiologic differences in sera from NPC patients from Hong Kong, America, Tunisia, and West Germany.

B. Activities of sera from Hodgkin's diseases and leukemia patients from Denmark and West Germany against various EBV antigenic determinants will be followed.

C. Serologic studies on activities of infectious mononucleosis (IM) sera against various EBV antigenic moieties will be performed.

D. Studies with HPA-23 will be made in relation to enhancement of MA in different EBV producer cell lines.

E. Studies will be performed on the biochemical and biological characterization of antigens resulting from alteration of the plasma membrane of human or simian cells transformed or infected by Epstein-Barr virus (EBV), and Herpesvirus ateles (HVA-810, HVA-73). Attempts will be made to increase HVA-810 titers for the above proposed MA studies.

Honors and Awards:

None

Publications:

Ablashi, D. V., Twardzik, D. R., Easton, J. M., Armstrong, G. R., Luetzeler, J., Jasmin, C., and Chermann, J-C.: Effects of 5-tungsto-2-antimoniate (HPA-23) in oncogenic DNA and RNA virus-cell systems. American Association for Cancer Research, San Diego, 1975.

Serial No. ZO1-CP-04846-03-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Viral Biochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Cellular expression of RNA-dependent DNA polymerase.

B. Characterization of defective RNA tumor virus isolates.

C. Studies of cell-virus interactions related to the Fv-1 locus.

Previous Serial Number: Same

Principal Investigator: Dr. Brenda I. Gerwin

Other Investigators: Dr. Robert H. Bassin

Cooperating Units: Inside NIH
Staff, VLL, NCI
Dr. Paul Peebles, VLL, NCI
Dr. George Vande Woude, VB, NCI

Outside NIH
Dr. Alan Rein, Litton Bionetics, Inc., Bethesda, Md.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To examine the nature of the RNA-dependent DNA polymerase expressed in human cells:

(1) On infection with a known RNA tumor virus (RD-114).

(2) In S+L- human cells and S+L- cells of other species to determine whether human cells are uniquely able to control the expression of this viral component.

B. (1) To examine the RNA of the noninfectious viruses produced by murine S+L- cells for homology to murine sarcoma and leukemia and utility as a probe for transforming sequences in cells.

(2) To characterize the biological defect of the noninfectious leukemia virus produced by some clones of superinfected S+L- cells. To attempt to correlate the biological defect with physico-chemical differences from infectious leukemia virus.

(3) To characterize noninfectious particles produced by clones of type B mouse cells after infection by N-tropic MuLV.

C. To determine whether murine sarcoma virus carries the information for N- or B-tropism as a stable genetic characteristic.

Methods Employed:

A. Affinity and ion exchange chromatography coupled with the use of antisera and various template/primer combinations and velocity gradient sedimentation were used to determine whether or not viral DNA polymerase was present in cells.

B. RNA from defective particles will be purified and characterized by CsCl extraction, velocity sedimentation, PAGE, and poly-U-sepharose chromatography. Nucleic acid hybridization will be used to determine the relationship of the sequences present in the defective virus particles to those in non-defective viruses.

C. Transformin virus rescued by N-, B-, or NB-tropic leukemia virus was assayed on N-, B- and dually permissive cells to determine the influence of helper leukemia on MSV tropism.

Major Findings:

A. (1) Cells which are producing RD-114 virus contain two forms of viral reverse transcriptase, one the normal form which is packaged in the virion and is 70,000 daltons, and a second of about 90,000 daltons which is a minor species in the infected cell and is apparently not packaged in the virion. The polymerases are identical antigenically.

(2) When MSV is rescued from human S+L- cells and used to reinfect mouse cells, clones are generated which produce particles containing reverse transcriptase. All S+L- clones of heterologous cells, e.g. human, dog, and mink, lack supernatant reverse transcriptase although murine p30 is present in all cells.

B. (1) Noninfectious particles produced by mouse S+L- cells contain a discrete high molecular weight RNA.

(2) The defective murine leukemia viruses produced by a clone of superinfected mouse S+L- cells can enter cells but are blocked within the cell. The particles, however, possess a quantitatively normal endogenous DNA polymerase reaction.

(3) Some of the noninfectious particles produced by infection of B-cells with N-tropic leukemia virus appear to have a high molecular weight RNA and normal reverse transcriptase. Others are extremely labile in density gradients and may be missing structural proteins.

C. The tropism of MSV is donated phenotypically by the rescuing leukemia virus.

Significance to Biomedical Research and the Program of the Institute:

A. (1) It is important to know the characteristics of reverse transcriptase in human cells. Since complete virus particles are a rarity in human cells, cellular markers for virus are especially important. The finding of a new form of reverse transcriptase which is associated with cells and may represent a precursor of the virion enzyme may be of diagnostic or predictive value in human cancer.

(2) These observations indicate that one reason for sarcoma defectiveness may be absence of functioning reverse transcriptase. This finding may be important in designing inhibitors of solid tumor formation.

B. (1) The RNA of these viruses may be a useful probe for sequences causing transformation which will aid in defining the mechanism of malignant transformation by sarcoma viruses.

(2 & 3) The isolation of noninfectious variants of murine leukemia viruses offers the possibility of defining genetic markers which will aid in understanding the life cycle of these viruses. In addition, the clones of cells which produce noninfectious viruses demonstrate a mechanism for control of infectious virus. Further definition of such mechanisms will aid in our ability to control the spread of type C virus.

C. The Fv-1 locus is known to be important genetically in the control of leukemia virus replication. The extension of our knowledge to sarcoma virus interactions at this locus will aid in our ability to induce or mimic cell control over type C virus.

Proposed Course:

A. These studies are completed.

B. In order to use these noninfectious virions as tools in genetic studies, we will attempt to correlate biological and biochemical defects in these classes of particles.

C. The availability of N- and B-tropic MSV will enable us to study with greater precision where, in the life cycle of the virus, the Fv-1 restriction acts.

Honors and Awards:

None

Publications:

Gerwin, B., Burstein, S. R., and Westley, J: Ascorbate Oxidase. Inhibition, Activation, and pH effects. J. Biol. Chem. 249: 2005-2008, 1974. 1974.

Papageorge, A. G., Peebles, P. T., Gerwin, B. I., Fischinger, P. J., and Mattern, C. F. T.: Expression of the defective "S+L-" type murine sarcoma virus genome in human amnion and lung cells. J. Nat. Cancer Inst. 52: 1727-1737, 1974.

Gisselbrecht, S., Bassin, R. H., Gerwin, B. I., and Rein, A.: Dual susceptibility of a 3T3 mouse cell line to infection by N- and B-tropic murine leukemia virus: Apparent lack of expression of the Fv-1 gene. Intl. J. Cancer 14: 106-113,1974.

Dunkel, V. C., Bast, Jr., R. C., Gerwin, B. I., Heine, U., Cottler-Fox, M., and Borsos, T.: Brief Communication: Presence of A-type and absence of C-type virus particles in a chemically induced guinea pig hepatoma. J. Nat. Cancer Inst. 53: 591-593, 1974.

Serial No. ZO1-CP-04848-03-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause
   and Prevention
2. Viral Biochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Studies on the mechanism of Fv-1 dependent cell-mediated resistance to murine leukemia viruses.

B. A study of the pathology of cloned murine sarcoma virus isolates in BALB/C mice.

C. Studies on S+L- cells superinfected with murine leukemia viruses.

Previous Serial Number: Same

Principal Investigator: Dr. Robert H. Bassin

Other Investigators: Dr. Brenda I. Gerwin
Dr. Graciela Duran-Troise
Dr. Martine Canivet

Cooperating Units: Inside NIH
Staff, VLL, NCI

Outside NIH
Dr. Alan Rein, Litton Bionetics, Inc., Bethesda, Md.
Dr. Jennifer J. Harvey, Clinical Research Centre, Harrow, England
Dr. Sylvie Gisselbrecht, Hopital Cochin, Paris

Man Years:

Total:	1.90
Professional:	1.00
Other:	0.90

Project Description

Objectives:

A. To study the mechanism of Fv-1 mediated cell resistance and susceptibility to murine leukemia and sarcoma viruses.

B. To study quantitatively the in vivo pathology of cloned stocks of several MSV isolates under uniform experimental conditions.

C. To examine the properties of replication defective variants of MuLV isolated after superinfection of S+L- cells.

Methods Employed:

Routine methods of in vitro propagation of cells and virus and selection of cloned lines of cells by soft agar and microplating techniques were employed. Quantitation of virus infection and transformation employed MSV assays in various mouse cells as well as the S+L- and XC assay techniques for leukemia virus infectivity.

Major Findings:

A. MSV pseudotypes produced after MuLV superinfection of nonproducer or S+L- cells display N-, B-, or NB-tropism similar to murine leukemia virus isolates. In all cases studied (Moloney S+L-, Moloney nonproducer, Kirsten, and Harvey) the tropisms of MSV pseudotypes were identical to those of the MuLV strains used for rescue. 3T3FL cells, which are dually susceptible to N- and B-tropic MuLV's are also equally susceptible to the corresponding MSV "pseudotypes". As is the case with resistance to tropic MuLV, resistance to MSV pseudotypes is not absolute, since foci are always found in nonproducer cells. A factor(s) which is responsible for MuLV susceptibility or resistance to Fv-1 restriction is apparently transferred to the MSV virion during the rescue process. This factor is not a stable part of the MSV genetic information and may be analogous to other phenotypically acquired properties, such as neutralizing antigens and host range. MSV "pseudotypes" are tropic when quantitatively assayed by techniques which measure either virus replication or cell transformation.

B. Several murine sarcoma virus strains have been isolated as cloned S+L- or nonproducer transformed cells derived from the 3T3FL cell line. These include MSV (Kirsten), MSV (Harvey) and MSV (Moloney - S+L-). Virus stocks have been prepared from each of these 3 transformed cell lines following superinfection with cloned B-tropic and NB-tropic MSV. All stocks were carefully titrated in tissue culture for both MSV and MuLV infectivity, and each stock was diluted to give a specified concentration of MSV and MuLV. These stocks have been inoculated into BALB/C mice of varying ages by Dr. J. J. Harvey, London, who is in the process of completing a series of comparative pathology studies.

C. Superinfection of S+L- cells with MuLV is usually followed by a morphological change and a cessation of cell division. A few cells survive the superinfection and continue to divide. Several cloned cell lines have been derived after MuLV superinfection of S+L- cells and one of these "8A", which exhibits a transformed morphology in culture, releases large numbers of type C virus particles which are biochemically similar to infectious MuLV. These particles are almost entirely noninfectious; when they are used to infect normal cells small amounts of progeny virus can be detected but only after a prolonged period in culture. These results suggest that some of the noninfectious particles from 8A cells contain a genome which can integrate

into normal cells but which cannot replicate until a further genetic alteration occurs.

Significance to Biomedical Research and the Program of the Institute:

A. Comparatively little is known at this time about the replication of RNA tumor viruses in host cells. Still less information exists about factors governing permissiveness and nonpermissiveness of cells from different species to various RNA tumor viruses (except for a few cases of cell surface associated phenomena). Control of RNA tumor virus infection by cells is an important area for investigation, not only for the question of virus replication but also as a means of understanding those factors which govern expression or repression of virus genetic information known to be present in most, if not all uninfected vertebrate cells. Fv-1 repression of MuLV in mouse cells appears to be a good system in which to study repression, since the mechanism of this repression has been shown not to involve virus absorption or penetration. The fact that MSV pseudotypes exhibit the same tropism as their MLV counterparts provides a new tool with which to study virus repression.

B. It is anticipated that certain in vivo properties characteristic of the murine leukemia sarcoma complex, such as splenomegaly in mice, will be assigned definitively to either MSV or MuLV information or to a combination of these two viruses. Differences among the MSV isolates will also be defined. Molecular hybridization studies have already indicated that the different isolates possess different genetic material and attempts will be made to correlate this difference with differences in in vivo properties.

C. Clone "8A" may represent a mutant which can perform some but not all of the functions of wild type MuLV. As such this mutant is of interest for genetic studies together with other MuLV mutants as well as for characterization of the nature of the defect. The fact that occasional infectious MuLV can be produced may also be of interest in studying the cellular control of repressed MuLV.

Proposed Course:

A. Studies are in progress to determine the biochemical and biological basis for the restriction of MSV pseudotypes in nonpermissive cells as well as for the ability of tropic MSV to overcome the restriction.

B. The pathological studies of mice infected with comparable titers of several MSV stocks will be completed and analyzed for significant differences among the MSV isolates.

C. The biology of defective MuLV clones will be studied and correlated with any immunological or biochemical deficiencies in the virions themselves.

Honors and Awards:

None

Publications:

Gisselbrecht, S., Bassin, R. H., Gerwin, B. I., and Rein, A.: Dual susceptibility of a 3T3 mouse cell line to infection by N- and B-tropic murine leukemia virus: Apparent lack of expression of the Fv-1 gene. Intl. J. Cancer 14: 106-113, 1974.

Rein, A., Gerwin, B. I., Bassin, R. H.: A replication-defective variant of MuLV. Bacteriol. Proc., in press.

Serial No. Z01-CP-04849-02-VLL
1. Viral Leukemia and Lymphoma Branch, OADVO, Division of Cancer Cause and Prevention
2. Primate Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on oncogenic herpesviruses of man and subhuman primates

Previous Serial Numbers: Same

Principal Investigator: Dr. John M. Easton

Other Investigators: Dr. Dharam V. Ablashi
Dr. Josef Luetzeler
Dr. Jean Guegan

Cooperating Units: Inside NIH
Dr. P. Levine, VLLB, NCI

Outside NIH
Dr. A. Liabeuf, International Agency for Research on Cancer, Lyon, France
Dr. G.B. de Thé, International Agency for Research on Cancer, Lyon, France
Dr. Claude Jasmin, Institut de Cancerologie et d'Immunogenetique, Villejuif, France
Dr. Daniel Twardzik, Frederick Cancer Research Center, Frederick, Maryland
Dr. Halvor Aaslestad, Frederick Cancer Research Center, Frederick, Maryland
Dr. Leigh J. Charamella, Frederick Cancer Research Center, Frederick, Maryland
Dr. Jean-Claude Chermann, Institut Pasteur, Paris, France
Dr. Jette Hesse, Institute of Medical Microbiology Copenhagen, Denmark

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To study the activities of sera from American Burkitt's lymphoma (AmBL) and Hodgkin's Disease (HD) patients against various Epstein-Barr virus (EBV) antigenic specificities, and to study the effect of the heteropolyanion 5-tungsto-2-antimoniate (HPA-23) on Herpesvirus saimiri (HVS) and EBV in vitro.

Methods Employed:

In studies of patients and control sera, a modification of the anticomplement immunofluorescence test of Reedman and Klein was used for the detection of nuclear antigen (NA) and antibody. A standard microtiter complement fixation (CF) test was employed for the detection of CF antibody. The CF antigen had a high particulate specificity. This same complement fixation test was also used for the titration of antibody to soluble (S) antigen. S antigen was prepared by standard cell disruptive and ultracentrifugal techniques. Early antigen(EA) was prepared by superinfecting Raji cells with EBV and acetone-fixing them on slides three days later. Viral capsid antigen (VCA) was prepared by maintaining Jijoye cells in an arginine-free medium. Tests were performed by standard methods.

In studies with HPA-23, human and simian cell cultures were maintained using standard techniques. Drug dilutions were made, and cell and direct virus toxicity were determined. Effect on virus replication was made by pretreatment and post-treatment of cells in relation to addition of virus. Titers were performed and calculated by standard techniques.

Major Findings:

Studies using S antigens showed close correlations between assays using antigens from 4 different lymphoblastoid cell lines (one from an AmBL patient, one from a nasopharyngeal carcinoma (NPC) patient, and 2 from African Burkitt's lymphoma (AfBL) patients). The sera used were from AmBL patients. Although there were occasional discordant sera, indicating that the antigens may not be identical, similar geometric mean titers (GMT) suggested more similarities than differences.

Analysis of the titers in AmBL and controls indicated that, in each of 4 test systems (EA, VCA, NA, CF), titers in AmBL patients were higher than those in control groups. In addition, the GMT for the 4 specificities of 10 patients who were still alive as of December 31, 1973 (3 of these patients long term survivors) suggested a positive correlation between antibody levels and survival. One patient was shown to seroconvert, during the course of the disease, in regard to 4 EBV-associated antibodies.

There was enhancement of HVS (1-1.5 log $TCID_{50}$/ml) in owl monkey kidney or Vero cells treated with HPA-23. This effect was noted when HVS was absorbed

2 hours prior to treatment with HPA-23, and also, to a lesser extent, by pretreatment with HPA-23 for 48 hours. Treatment of a low VCA producer (1%) line of P3HR1 cells with HPA-23 induced approximately 15% VCA and EA. HPA-23 also stimulated the growth of B lymphocytes (P3HR1 and HK-Ly-28 cells). HPA-23 did not show any significant enhancement of VCA in P3HR1 cells already producing VCA ($\geq$ 10-15%).

Three hundred sera from HD patients from Denmark, and controls, have been titrated against EBV S and nuclear antigens. These sera are all coded. A crude GMT of 28 to NA and 9 to S were found. The titers were much lower than those of NPC, and will yield more meaning when the patient relationship is uncoded.

Significance to Biomedical Research and the Program of the Institute:

The serologic data suggest a possible relationship between EBV and AmBL, as previously reported by us and others, but this is more subtle than that for AfBL, NPC, and infectious mononucleosis, because EBV genomes have not been detected in a majority of biopsies of EBV tumors. While multiple etiologies and difficulties in making a specific diagnosis can cause some confusion in studies of BL, it is still possible to state that cases of AmBL share many of the clinical and laboratory features of AfBL. The finding that the immune response to the EBV S antigen is quite similar, although the antigens are prepared from different cell lines suggests that the viruses associated with AfBL and AmBL are similar, but the discordancies found in some sera indicate that there may be some differences. These, if they do exist, may be identified biochemically when the antigens are studied in a purified form.

HPA-23 has been shown by Jasmin et al. to protect mice against Friend and plasma variant-induced leukemias as well as to delay tumor induction by Moloney murine sarcoma virus. HPA-23 has a broad and potent antiviral activity, and its inhibition of multiplication of RNA and DNA non-oncogenic viruses was quite consistent. Studies of the effect of HPA-23 on HVS and EBV may throw some light on its complex mechanism of action.

When the results dealing with the HD sera are complete, further conclusions would be able to be drawn about the relationship of EBV to HD from an immunologic point of view.

Proposed Course:

AmBL patients and their tumors will be studied in order to determine the relationship, if any, of EBV to the disease. It is possible that tumor biopsies will be studied as to their content of nuclear antigen.

We hope to determine whether HPA-23 could enhance infectious EBV as it did HVS. We also hope to continue studying the effect of HPA-23 on enzyme systems which may be involved in the suppression or enhancement of virus replication.

Antibody levels to EBV S and nuclear antigens will be correlated with the clinical status of the HD cases and attempts will be made to draw conclusions in regard to the relationship of clinical status to antibody level.

Honors and Awards:

None

Publications:

Ablashi, D. V., Armstrong, G. R., Easton, J. M., Chopra, H. C., and Adamson, R. H.: Evaluation of cytosine arabinoside on Herpesvirus saimiri replication in owl monkey kidney cells. Biomed. Express 21: 57-60, 1974.

Ablashi, D. V., de Thé, G. B., Easton, J. M., Liabeuf, A., Levine, P. H., and Armstrong, G. R.: Antibodies to Epstein-Barr virus (EBV) antigens in sera of American Burkitt lymphoma patients. Biomed. 20: 288-293, 1974.

Easton, J. M., Ablashi, D. V., de Thé, G. B., Levine, P. H., Twardzik, D. R., Aaslestad, H. G., and Armstrong, G. R.: Association of Epstein-Barr virus (EBV) and American Burkitt's lymphoma (AmBL). In de Thé, G., Epstein, M. A., and zur Hausen, H. (eds.): Oncogenesis and Herpesviruses II. Lyon, France. International Agency for Research on Cancer, WHO (in press).

Didier, M. L., Ablashi, D. V., Oie, H. K., Armstrong, G. R., Easton, J. M., Chu, E., and Rabson, A.: Some biological properties of Herpesvirus saimiri from chronically infected monolayer and suspension cultures. In de Thé, G., Epstein, M. A., and zur Hausen, H. (eds.): Oncogenesis and Herpesviruses II. Lyon, France. International Agency for Research on Cancer, WHO, (in press).

Ablashi, D. V. and Easton, J. M.: Approaches to a preventive vaccine against Herpesvirus saimiri-induced neoplasia. Symposium on Immunologic Control of Virus-associated Tumors in Man: Prospects and Problems. Bethesda, Maryland, 1975 (in press).

Serial No. ZO1-CP-04850-03-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Reversion of mouse cells transformed by MSV

Previous Serial Number: Same

Principal Investigator: Dr. Shigeko Nomura

Other Investigators: Dr. Peter J. Fischinger
Dr. Daniel K. Haapala
Dr. Paul T. Peebles
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Dr. Janet W. Hartley, LVD, NIAID
Mr. Paul R. Hill, VC, NCI
Dr. Carl F. T. Mattern, LVD, NIAID

Outside NIH
None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To study the effect of chemicals on the frequency of reversion of the Kirsten MSV (Ki-MSV)-transformed BALB/3T3 (Ki-BALB) cells to flat variants and to compare the biological characteristics of flat variants isolated either spontaneously or chemically.

B. To study the interactions between endogenous oncornavirus genomes and the exogenous MSV genomes in cells undergoing sequential cycles of reversion and retransformation.

Methods Employed:

Standard tissue culture and virological procedures were used. Chemical treatments were carried out by exposing cells to 10 µg/ml of FdU for 1 hour or 0.04 µg/ml of colcemid for 24 hours. Induction of MSV or MuLV was performed by treating cells with 20 µg/ml of IdU for 30 hours and cell cocultivation.

Major Findings:

A. The Ki-BALB cells spontaneously produced flat variants with some properties of nontransformed cells at frequences of 0.01 to 0.001. Such variants were epithelioid, contact-inhibited, grew to low saturation density and exhibited variable cloning efficiencies in soft agar. They demonstrated no MuLV gs antigen(s), no reverse transcriptase activity, and no infectious virus, but were agglutinable by concanavalin A. MuLV was induced after IdU treatment from both the flat variants and from the parental Ki-BALB cells. However, Ki-MSV could not be rescued from these flat variants by superinfection with MuLV, nor induced by treatment with IdU. The state of reversion was stable, revealing no back-transformation during 30 to 40 subcultures. The tumorigenicity of the flat variants in BALB/c mice was markedly reduced (actually undetectable) compared to that of the Ki-BALB cells. All flat variants were susceptible to MSV and MuLV infection. The loss of the transformed phenotype in spontaneous flat variants was associated with a decrease in chromosome number to a level similar to BALB/3T3 cells.

B. Retransformation of S+L- revertants by Moloney MSV resulted in a number of metastable states, and no second cycle of reversion was observed. The S+L- revertants retransformed Ki-MSV demonstrated a rescuable and inducible MSV, and a second cycle of reversion occurred in these cases. The NRK cells transformed by MSV, which has been rescued from retransformed S+L- revertants by Ki-MSV, demonstrated MuLV gs antigen in 2 out of 11 clones isolated in the absence of infectious virus.

Significance to Biomedical Research and the Program of the Institute:

Studies of the expression of virus information in our systems and the effect of chemical agents on reversion will increase our understanding of viral oncogenesis and basic cellular regulatory controls.

Proposed Course:

Further biological and biochemical studies of the reversion process.

Honors and Awards:

None.

Publications:

Nomura, S., and Fischinger, P. J.: Control of reversion in MSV transformed cells after single and multiple transforming cycles. In Robinson, W. S. and Fox. C. F. (Eds.): Mechanisms of Virus Disease. Menlo Park, Calif., W. A. Benjamin, Inc., 1974, pp. 273-285.

Fischinger, P. J., Nomura, S., and Peebles, P. T.: Control of reversion of MSV transformed cells. In Ito, Y. and Dutcher, R. (Eds.): Comparative Leukemia Research 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 475-481.

Nomura, S., Dunn, K. J., Mattern, C. F. T., Hartley, J. W., and Fischinger, P. J.: Revertants of mouse cells transformed by murine sarcoma virus: flat variants without a rescuable sarcoma virus from a clone of BALB/3T3 transformed by Kirsten MSV. J. Gen. Virol. 25: 207-218, 1974.

Fischinger, P. J., Nomura, S., Tuttle-Fuller, N., and Dunn, K. J.: Revertants of mouse cells transformed by murine sarcoma virus. III. Metastable expression of virus functions in revertants retransformed by murine sarcoma virus. Virology 59: 217-229, 1974.

Fischinger, P. J., Blevins, C. S., and Nomura, S.: Simple, quantitative assay for both xenotropic murine leukemia and ecotropic feline leukemia viruses. J. Virol. 14: 177-179, 1974.

Fischinger, P. J., and Nomura, S.: Efficient release of murine xenotropic oncorna virus after murine leukemia virus infection of mouse cells. Virology, in press.

Fischinger, P. J. and Nomura, S.: Alteration of malignancy in cultured cells. Proceedings, XI International Cancer Congress. Amsterdam, Excerpta Medica, in press.

Serial No. ZO1-CP-04853-03-VLL
1. Viral Leukemia and Lymphoma Branch
OADVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Isolation and characterization of mammalian type C viruses

Previous Serial Number: Same

Principal Investigator: Dr. Raoul E. Benveniste

Other Investigators: Dr. Robert Callahan
Dr. Charles J. Sherr
Dr. George J. Todaro

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	2.5
Professional:	1.0
Other:	1.5

Project Description

Objectives:

To isolate infectious type C viruses from various mammalian species from which such viruses have not yet been isolated and to study the relationships among these viruses primarily by comparing their nucleic acid sequence homology.

Methods Employed:

New type C viruses are isolated by cocultivation of cell lines and tissues from various mammalian species with a wide variety of cell lines. The presence of a type C virus replicating in a cell line is usually first detected by assaying a high speed pellet of tissue culture media with synthetic templates that can detect low levels of reverse transcriptase activity. Specific antisera prepared against all the known mammalian type C viral reverse transcriptases and radioimmunoassays for various type C viral proteins are employed to characterize new isolates and to test various tissue extracts and cell lines for translation of viral proteins. Radioactively-labeled ^{3}H-DNA

copies of the various type C viral genomes are available to examine for the presence of related nucleic acid sequences in cell lines and tissues of various species and to characterize the species of origin of new isolates. A wide variety of virologic assays are also used to study the biologic properties of new type C isolates, such as host range, viral interference patterns, and presence of transforming (sarcoma) viruses.

Major Findings:

(A) Human tissues contain endogenous primate type C virogene information.

Endogenous type C viruses have been isolated from three different baboon species (genus Papio) by cocultivation of various tissues and cell lines with suitable heterologous mammalian cells. These viruses are distinctly different by immunologic and nucleic acid hybridization techniques from previously studied mammalian type C viruses. With ^{3}H-DNA transcripts prepared from the endogenous baboon type C viruses, partially homologous sequences have been detected in the cellular DNA of all species of the primate suborder Anthropoidea (Old World monkeys, apes, and man). This demonstrates that all Old World monkey and great ape families (including the human family Hominidae) possess endogenous type C virogenes, and, thus, the potential for producing endogenous type C viruses. Within the primates, type C viral genes have evolved as the species have evolved, with virogenes from more closely related genera and families having more sequence homology than those from distantly related taxons. The ubiquitous presence of endogenous type C virogenes among anthropoid primates and their evolutionary preservation for at least 30-40 million years suggest that such genes provide functions with a selective advantage to the species possessing them.

(B) Evidence for interspecies transfer of type C viruses.

By several criteria, including nucleic acid sequence homology and antigenicity of the polymerase and the p30 protein, the various baboon viruses are related to, but distinct from, the endogenous cat viruses of the RD-114/CCC group. The unexpected relatedness of the endogenous cat and the endogenous baboon viruses suggested that they had a common origin. All members of the cat family Felidae (36 species belonging to six genera) are closely related as shown by hybridization of their cellular DNA. Only four species of the genus Felis (domestic cat, sand cat, jungle cat, and European wildcat) contain endogenous virogenes related to the RNA of RD-114. However, all Old World monkey and ape species examined have nucleic acid sequences related to RD-114. These findings suggest that viral genes from primates gave rise to infectious particles that not only integrated into the DNA of an ancestor of the domestic cat but were incorporated into the germ line and transmitted as cellular genes. By examining the divergence time of the various species discussed above, this infection is believed to have occurred about 5-7 million years ago in the Mediterranean basin.

By molecular hybridization, a strong nucleic acid sequence relationship was also shown between the pig type C viral ^{3}H-DNA probes and murine cellular DNA.

This unexpected relationship again suggested that these two viruses had a common origin. A comparison of various rodents and ungulates for virogenes related to the known mouse and pig viruses suggested that the pig virus arose by infection of an ancestor of the domestic pig with a murine type C virus approximately seven million years ago.

The comparison of the nucleic acid sequences of all mammalian type C viruses has likewise also led to the observation that the feline leukemia virus arose as an infection from rodents with a type C virus. The infectious primate type C viruses isolated from a woolly monkey and a gibbon ape have been shown to be related immunologically and by nucleic acid hybridization to murine viruses; these data again are consistent with the possibility that these viruses arose from endogenous murine viruses. The woolly monkey and gibbon ape type C viruses, however, are not endogenous primate viruses and have only been isolated from a few gibbon apes and one woolly monkey.

(C) Endogenous mammalian viruses are present as a family of multiple partially related viruses in their species of origin.

It is characteristic of endogenous type C viruses that all individual animals of a species, as well as all tissues of each animal, contain, in the chromosomal DNA, nucleic acid sequences that can code for the production of complete type C viral particles. We examined, by molecular hybridization, the reiteration frequency of mouse, rat, pig, cat, and baboon viral nucleic acid sequences in tissues and cell cultures of the species of origin of these viruses and in exogenously infected cells of heterologous species. While DNA from each species contains multiple (5-15) copies of endogenous virogene sequences per haploid genome, cell lines of heterologous species infected with and producing high titers of these type C viruses contain fewer copies (one or two per haploid genome). The thermal stability of DNA:DNA hybrids demonstrates that the endogenous virogenes are composed of families of genes with related but not identical nucleic acid sequences. The presence of multiple copies of viral information is thus a useful criterion with which to identify the species of origin of an endogenous mammalian type C virus. Whether during their evolution as cellular genes the virogenes of a single species have diverged from one another in function, as they have in structure, remains to be determined.

Significance to Biomedical Research and the Program of the Institute:

The cocultivation techniques which have resulted in the isolation of endogenous primate type C viruses from baboons are applicable to the isolation of a variety of mammalian type C viruses.

We have shown that all higher primates, including man, contain information in their tissues (antigens and nucleic acid sequences) related to endogenous primate type C viruses.

The discovery that many of the mammalian type C viruses arose by transpecies infection shows that viruses of one species can readily infect another species

in vivo.

Molecular hybridization techniques can be used to predict the species from which type C viruses can be isolated. This was demonstrated for the European wildcat (Felis sylvestris) from which a type C virus was isolated that, as predicted from hybridization experiments, was closely related to viruses of the RD-114/CCC group.

Proposed Course:

We are attempting to isolate new type C viruses, especially from primate and human tissues. The apparent generality of transpecies infections of type C viruses raises the possibility that these viruses may cause neoplasia when they are in the process of being transferred from one species to another. Since there are many closely related animals available that either possess or lack virogene sequences, this possibility is being actively investigated by inoculating the appropriate animals with type C viruses.

Honors and Awards:

None

Publications:

Benveniste, R.E., Lieber, M.M., Livingston, D.M., Sherr, C.J., Todaro, G.J., and Kalter, S.S.: Infectious type C virus isolated from a baboon placenta. Nature 248: 17-20, 1974

Sherr, C.J., Lieber, M.M., Benveniste, R.E., and Todaro, G.J.: Endogenous baboon type C virus (M7): Biochemical and immunological characterization. Virology 58: 492-503, 1974.

Todaro, G.J., Sherr, C.J., Benveniste, R.E., Lieber, M.M., and Melnick, J.M.: Type C viruses of baboons: Isolation from normal cell cultures. Cell 2: 55-61, 1974.

Benveniste, R.E., Heinemann, R., Wilson, G.L., Callahan, R., and Todaro, G.J.: Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. J. Virol. 14: 56-57, 1974.

Sherr, C.J., Benveniste, R.E., and Todaro, G.J.: Type C viral expression in primate tissues. Proc. Nat. Acad. Sci. U.S.A. 71: 3721-3725, 1974.

Benveniste, R.E., and Todaro, G.J.: Multiple divergent copies of endogenous C-type virogenes in mammalian cells. Nature 252: 170-173, 1974.

Benveniste, R.E., and Todaro, G.J.: Evolution of type C viral genes. I. Nucleic acid from baboon type C virus as a measure of divergence among primate species. Proc. Nat. Acad. Sci. U.S.A. 71: 4513-4518, 1974.

Benveniste, R.E., and Todaro, G.J.: Evolution of C-type viral genes: inheritance of exogenously acquired viral genes. Nature 252: 456-459, 1974.

Callahan, R., Benveniste, R.E., Lieber, M.M., and Todaro, G.J.: Nucleic acid homology of murine type C viral genes. J. Virol. 14: 1394-1403, 1974.

Benveniste, R.E., Sherr, C.J., Lieber, M.M., Callahan, R., and Todaro, G.J.: Evolution of primate type C viral genes. In Gottlieb, A. Arthur (ed.): Fundamental Aspects of Neoplasia. New York, N.Y., Springer-Verlag, 1975.

Todaro, G.J., Benveniste, R.E., Callahan, R., Lieber, M.M., and Sherr, C.J.: Endogenous primate and feline type C viruses. In Cold Spring Harbor Symposium on Quantitative Biology XXXIX. The Tumor Viruses. Cold Spring Harbor, N.Y., Cold Spring harbor Press, 1975.

Lieber, M.M., Benveniste, R.E., Sherr, C.J., and Todaro, G.J.: Isolation of a type C virus (FS-1) from the European wildcat (Felis sylvestris). Virology (In press).

Sherr, C.J., Fedele, L.A., Benveniste, R.E., and Todaro, G.J.: Interspecies antigenic determinants of the reverse transcriptases and p30 proteins of mammalian type C viruses. J. Virol. (In press).

Lieber, M.M., Benveniste, R.E., Sherr, C.J., and Todaro, G.J.: Endogenous porcine type C viruses. Virology (In press).

Serial No. Z01-CP-04858-03-VLL
1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause
   and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A. Mechanism of transformation by RNA tumor viruses
B. Transcriptional control of endogenous RNA tumor viruses

Previous Serial Number: Same

Principal Investigator: Dr. Edward M. Scolnick

Other Investigators: Dr. Andrew Silverman

Cooperating Units: Inside NIH
Dr. Wade Parks, VC, DCCP, NCI
Dr. Arthur Levine, PO, NCI

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia and Rockville, Maryland
University of California, Berkeley California

Man Years:

Total:	5.0
Professional:	2.0
Other:	3.0

Project Description

Objectives:

A. We have investigated the mechanism of transformation of RNA tumor viruses. The ultimate goal is to identify in a human cancer cell, a protein coded for by an RNA tumor virus responsible for the cancerous state of that cell. In order to study this phenomenon we have studied RNA-containing sarcoma viruses of mammalian species.

B. The second major effort of the Genetics Section has been to focus on developing an in vitro biochemical assay for studying the transcription of endogenous RNA tumor virus genomes and thus provide a mechanism by which we could identify the epigenetic and genetic mechanisms involved in the control of transcription of RNA tumor viruses. In the past year we have developed in collaboration with Dr. Wade Parks of the VCB a comprehensive effort at

studying in vitro the transcription control of RNA tumor viruses.

Methods Employed:

A. For the transformation studies the methods involve a series of tissue culture assays in order to isolate the various nonproducer cells, including soft agar, cloning of cells in microtest II plates, the establishment of a series of nonproducer cells of the mammalian sarcoma viruses where the sarcoma virus genome is present in many different species of mammalian cells. The biochemical methods involve the use of sl nuclease for hybridization assays, and the synthesis of endogenous DNA transcripts from the endogenous reaction of RNA tumor viruses. The more recent investigations of the nature of the rat genetic information involve the fractionation of RNA by size in acrylamide gels and on sucrose gradients, the preparation of polysomes from cells, and the use of various DNA-DNA hybridization techniques such as sl, hydroxyapatite, or other solid phase assays.

B. The methods involved in the transcription problem are varied and comprehensive. They involve the purification of enzymes involved in transcription by DEAE, phosphocellulose, Sephadex, and hydroxyapatite column chromatography. They involve the preparation of cellular chromatin by a series of salt elutions and sedimentations. They involve the fractionation by column chromatography and glycerol gradient sedimentation of the cellular chromatin into active "euchromatin" and inactive "heterochromatin" fractions. They have required the purification from bacterial cells as tools, RNase III an enzyme specific for double stranded RNA degradation, and RNase H an enzyme specific for the degradation of an RNA strand of an RNA-DNA hybrid.

Major Findings:

A. The major findings in the study of the mechanism of transformation involve the first formal proof that RNA tumor viruses can undergo stable genetic recombination and that the oncogenic information contained in fibroblast transforming type C viruses may not be type C in origin but be cellular in origin. This is the first formal proof that transduction of genetic information can occur in mammalian systems.

1. We have isolated nonproducer cells containing the sarcoma viral genome of the Moloney sarcoma virus, the Kirsten and Harvey strains of sarcoma virus, several strains of Feline sarcoma virus, and woolly monkey sarcoma virus. We have succeeded in isolating such nonproducer cells for all strains of mammalian sarcoma virus excluding the FBJ strain of murine sarcoma virus.

2. No proteins are known yet which are coded for by these transforming agents, and each of these agents is defective for replication. Therefore, we have studied by nucleic acid hybridization the genetic information contained in all of these RNA sarcoma viruses. We have shown that in fact mammalian RNA sarcoma viruses are type C viruses because they contain as part of their genome, nucleic acid which is homologous to replicating type C viruses. This is true in each case of the above named sarcoma viruses. Interestingly, the

woolly monkey sarcoma virus contains information homologous to the woolly leukemia virus and this information is more closely related to the woolly leukemia virus than to any of the known gibbon ape leukemia viruses. The feline sarcoma viruses contain feline leukemia information but no information homologous to another feline virus RD114. The murine sarcoma viruses contain nucleic acid information homologous to the original leukemia virus present in the stock used to isolate the virus.

3. In order to explore the mechanism by which these viruses transform fibroblasts we have looked for additional sequences since the leukemia sequences present in the virus cannot explain in a simple way the ability of these viruses to transform fibroblasts since none of the homologous leukemia viruses have this property. We have studied in greatest detail up to now the Kirsten strain and Harvey strain of sarcoma virus with this end in mind. The Kirsten sarcoma virus was isolated by passage of Ki-MuLV in rats, and the Harvey sarcoma virus by passaging the Mo-MuLV in rats. We have identified in these two sarcoma viruses genetic information not present in either the Kirsten strain of leukemia virus or the Moloney strain of leukemia virus. The additional information present in the Kirsten and Harvey viruses comes from rat cells and is present in normal rat cellular DNA. Apparently, the rat genetic information present in Kirsten and Harvey sarcoma viruses is the same. Thus, in two independent isolates of transforming viruses a gain of rat genetic information has occurred. The nature of the rat genetic information has been extensively studied in the past year and in fact an exciting observation has been that the rat genetic information in these two sarcoma viruses is distinct from the known rat type C viral nucleic acid sequences and is present in normal rat cells. The question is unresolved as to whether the new rat genetic information is of cellular origin or represents a distinct type C virus in rats different from the known rat type C viruses. This is a very important question to resolve in future experiments because there is abundant biological evidence to suggest that the rat information gained during the passage of the two mouse viruses in rats accounts for the information responsible for the maintenance of the transformed state in these cells. If the information is of cellular and not type C origin then the transforming information initially would be considered nonviral. If the information is type C then this would provide direct experimental proof that type C viruses can code for transforming information. This cannot be directly proved for any leukemia producing virus. The nature of the rat genetic information from cells is being explored in great detail and the RNA that is present in some normal nontransformed rat cells homologous to this rat genetic information is being characterized in order to further elucidate its nature and to provide means of isolating the protein for which this RNA codes. Presumably this would be the protein responsible for the maintenance of transformation.

4. In collaboration with Dr. Peter Duesberg of the Virus Cancer Program from the University of California in Berkeley, we have been able to show that the mouse and rat information contained in the Harvey sarcoma virus are covalently linked. These experiments directly prove in a way that has not been possible up to now that RNA tumor viruses are capable of undergoing classic type of genetic recombination by crossing-over which leads to covalent linkage of distinct genetic information. Although abundant indirect evidence by Duesberg

and Vogt has been present to suggest this up to now, these experiments directly prove that RNA tumor viruses can undergo genetic recombination and provide an important model for how a potentially non-oncogenic RNA containing type C virus can become an oncogenic RNA type C virus. These studies are being extended to RNA containing leukemia viruses in order to attempt to identify the genetic information in RNA leukemia viruses which could code for production of leukemia.

B. The major findings in the transcriptional control problem relate to a) the mechanism of RNA polymerase II in which it has been shown that its method of transcription of chromatin is different from RNA polymerase I, b) the purification of protein factors which are important in regulating the transcription of chromatin by RNA polymerase II, c) the application of an exceedingly sensitive hybridization assay for the detection of type C nucleic acid sequences in cells, which has approximately a 50-100 fold greater sensitivity than previous hybridization assays, d) the preliminary findings on the nature of the dexamethasone receptor in mouse cell which indicate that sulfhydryl groups are involved in the active site of the receptor which is important for the induction of type B viruses. Further information about the active site could lead to chemicals which could potentially inactivate a receptor and have implications for the treatment of type B virus induced tumors.

1. We have purified from mouse cells mammalian RNA polymerase I and mammalian RNA polymerase II. Mammalian RNA polymerase II is thought to be involved in the transcription of DNA proviral material of RNA tumor viruses and we have studied the mechanism by which mammalian RNA polymerase II works in general in transcribing DNA and in particular the RNA tumor virus genome. The general mechanism of mammalian RNA polymerase II is being studied on synthetic and natural templates, and a project has been begun with Dr. Arthur Levine, PO, NCI, to study the transcription of the adenovirus genome which is a simple and well-characterized oncogenic DNA virus which can provide a simpler model system to give us information about the mechanism of action of the polymerase itself. These studies will provide important background information for the study of the more complex transcription of the cellular DNA containing the RNA tumor virus genome.

2. We have purified from mammalian cells, protein factors which greatly enhance the transcription by mammalian RNA polymerase II of native DNA from cells, and adenovirus native DNA. The nature and number of these protein factors and their mechanism of action is being investigated. It is not known whether these factors play a role in initiation of transcription or in the elongation of transcription.

3. We have developed methods to study the transcription of cellular chromatin containing proviral genetic information and investigated the structure of chromatin in ways not possible before by the use of RNA tumor viruses. We have investigated in detail the genetic information contained in fractions of chromatin which can be obtained from cells which are the in vitro counterpart of euchromatin and heterochromatin. By using the RNA tumor viruses as

markers for genetic information in euchromatin and heterochromatin, we have been able to obtain abundant new information about the basic structure of cellular chromatin and provide important background information for studying the regulation of transcription of RNA tumor viruses. We have found, in contrast to what abundant information in the field would have led one to believe, that in vitro fractionated eu- and heterochromatin contain equal amounts of inactive and active genetic information. The implication of these findings for future studies on the transcription and structure of chromatin are very important. The results imply that the structure of the mammalian genome is much more similar to simple pro-karyotic organisms than has heretofore been believed.

4. We have developed an exceedingly sensitive hybridization assay to allow us to detect in transcripts from chromatin as little as 0.02% of the transcript as type C viral or type B viral specific. This in vitro assay has profound use in studying transcription from chromatin and can also potentially be used in trying to identify, in transiently pulse labelled cells, type C information whose turnover in cells might be too fast to identify it by the more typical hybridization assays which we have previously described.

5. In collaboration with Dr. Parks of the Viral Carcinogenesis Branch, we have studied the steroid hormone receptor of the mouse cell and developed in vitro assays for its purification and identification in order to study its relationship to the stimulation of type B viral RNA production.

Significance to Biomedical Research and the Program of the Institute:

A. It has become exceedingly clear in the past few years that not all RNA-containing, reverse transcriptase containing viruses are oncogenic. In addition, it is clear that some RNA tumor viruses clearly are oncogenic and that the known structural proteins of RNA tumor viruses such as the gs antigen or the reverse transcriptase cannot predict the oncogenicity of a given strain. The studies on RNA sarcoma virus provide a method for identifying the genetic information of the RNA tumor virus that is involved in oncogenicity and provide a means by which we can eventually identify the protein involved in transformation by RNA tumor viruses. It is not known for any oncogenic virus, whether DNA or RNA, what protein is responsible for the maintenance of transformation by that virus. The systems developed in our laboratory on the study of the Kirsten and Harvey strains of sarcoma virus provide what is probably currently the best experimental system of any oncogenic virus to attempt to identify in the near future the protein responsible for the maintenance of transformation. Such a discovery would be of fundamental importance both to human cancer and to the understanding of the malignant process in general.

B. The process designed to study the transcription of endogenous RNA tumor virus genetic material is important from the point of view that if RNA tumor viruses are transmitted vertically and are important in determining cancer, and this has certainly been proved in certain animal systems, it may be difficult to prevent the disease by standard vaccination methods in these cases. Eventually one needs to know more about how the endogenous viruses are

regulated in order to be able to prevent the turn-on of a virus and its oncogenic action. The development of in vitro systems in which we can study this regulation should allow this to be possible.

Proposed Course:

A. The sarcoma virus project will continue to extend the above information. The project will focus on characterizing in great detail the rat genetic information found in Kirsten and Harvey sarcoma virus which is apparently necessary for the maintenance of transformation. The methods used for identifying oncogenic information in the murine sarcoma viruses will attempt to be extended to both Feline and Primate sarcoma viruses in an attempt to compare the oncogenic information from a variety of species of mammalian RNA sarcoma viruses. Attempts will be made to identify immunologically the protein coded for by RNA sarcoma viruses responsible for the maintenance of transformation. These studies will be done in collaboration with Dr. Wade Parks of the Viral Carcinogenesis Branch.

B. The proposed course of the transcriptional problem will be to begin to study in detail now the transcription of type C genetic information in mouse cell systems now that the assays have been developed over the past year to do this. In addition, we will continue to study the transcription of the adenovirus genome as a simpler model system for learning in greater detail about the enzymes involved in mammalian transcription. We will attempt toward the end of the next year to more fully extend the systems to the type B system and in particular to coordinate our efforts with Dr. Parks on the effect of dexamethasone on the transcription of type B genetic information. Technically, the type B system is more difficult currently and we are initiating our experiments in the type C system and the adenovirus system with the future to be extended to the type B system once greater intellectual and technical insights have been gained into the transcriptional systems involved.

Honors and Awards:

Election to the American Society for Biological Chemists

Publications:

Scolnick, E.M. and Parks, W.P.: Host range studies on xenotropic type C viruses in somatic cell hybrids. Virology 59: 168-178, 1974.

Scolnick, E.M., Parks, W.P.: Harvey sarcoma virus: A second murine type C sarcoma virus with rat genetic information J. of Virol. 13: 1211-1219, 1974.

Parks, W.P., Howk, R.S., Scolnick, E.M., Oroszlan, S. and Gilden, R.V.: Immunochemical characterization of two major polypeptides from murine mammary tumor virus. J. of Virol. 13: 1200-1210, 1974.

Parks, W.P. and Scolnick, E.M.: Immunological cross-reactions between two low molecular weight polypeptides from a murine type C virus. J. of Virol. 14: 430-433, 1974.

Howk, R.S., Williams, D.R., Haberman, A.B., Parks, W.P. and Scolnick, E.M.: A comparison of the transcription of mouse cell chromatin by homologous RNA polymerase I and II. Cell 3: 15-22, 1974.

Scolnick, E.M., Goldberg, R.J. and Parks, W.P.: A biochemical and genetic analysis of mammalian RNA containing sarcoma viruses. Cold Spring Harbor Tumor Virus Symposium, 39: 1974, in press.

Parks, W.P., Scolnick, E.M., and Ransom, J. C.: Glucocorticoid induction of murine mammary tumor virus in vitro. Cold Spring Harbor Symposium, 39: 1974, in press.

Scolnick, E.M., Maryak, J.M. and Parks, W.P.: Levels of rat cellular RNA homologous to either Kirsten sarcoma virus or rat type C virus in cell lines derived from Osborne-Mendel rats. J. of Virol., 14: 1435-1444, 1974.

Goldberg, R.J., Scolnick, E.M. and Parks, W.P.: Isolation of a primate type C virus from a lymphomatous baboon. Internat. J. of Cancer, 14: 722-730, 1974.

Parks, W.P., Ransom, J.C., Young, H.A. and Scolnick, E.M.: Mammary tumor virus induction by glucocorticoid: characterization of specific transcriptional regulation. J. of Biol. Chem., in press.

Young, H.A., Scolnick, E.M. and Parks, W.P.: Glucocorticoid-receptor interaction and induction of murine mammary tumor virus. J. of Biol. Chem., in press.

Howk, R.S., Anisowicz, A., Silverman, A.Y., Parks, W.P. and Scolnick, E.M.: Distribution of murine type B and type C viral nucleic acid sequences in template active and template inactive chromatin. Cell, in press.

Serial No. Z01-CP-04864-03-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause
   and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunological investigations of virus-induced tumors

Previous Serial Number: Same

Principal Investigator: Dr. Tadao Aoki

Other Investigators:
Dr. Vincent W. Hollis, Jr.
Dr. Ernest J. Plata
Dr. Phyllis B. Brandchaft
Dr. Etsuo Okazaki
Dr. Hideki Kimura

Cooperating Units:

Inside NIH
Dr. Stuart Aaronson, VC, NCI
Dr. Kenneth S.S. Chang, LCBGY, NCI
Dr. Peter Fischinger, VLLB, NCI
Dr. Ronald Herberman, LCBGY, NCI
Dr. Michael Potter, LCBGY, NCI
Dr. John Stephenson, VC, NCI
Dr. Kenneth K. Takemoto, LVD, NIAID

Outside NIH
Dr. Lloyd J. Old, Memorial Sloan-Kettering Cancer Center, New York, New York
Dr. Edward A. Boyse, Memorial Sloan-Kettering Cancer Center, New York, New York
Dr. Hidetoshi Sato, Memorial Sloan-Kettering Cancer Center, New York, New York
Dr. Hidesaburo Hanafusa, The Rockefeller University, New York, New York
Dr. Luigi Chieco-Bianchi, University of Padova, Padova, Italy
Dr. Harry A. Wood, Boyce Thompson Institute for Plant Research, Yonkers, New York
Dr. Frank J. Dixon, Scripps Clinic and Research and Foundation, La Jolla, California
Dr. Michael B.A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California

Dr. Roger E. Wilsnack, Huntingdon Research Center, Baltimore, Maryland
Dr. Konrad C. Hsu, Columbia University, New York New York
Dr. Stephen Oroszlan, Flow Laboratories, Rockville, Maryland
Dr. Raymond V. Gilden, Flow Laboratories, Rockville Maryland

Man Years:

Total:	5.0
Professional:	1.0
Other:	4.0

Project Description

Objectives:

A. To analyze the specificity of mammalian sarcoma type C virus-induced cell surface antigens.

B. To determine the relationship between pristane and Abelson's virus in plasmacytoma-induction.

C. To study the effect of lyophilized Streptococcus hemolyticus on type C virus-replication.

D. To prove the heterogeneity of endogenous type C viruses by the specificity of surface antigens.

E. To identify a new surface antigen (MEV-SA1) induced by a certain endogenous type C virus.

F. To demonstrate the role of blood platelets in the growth of type C virus-induced tumors.

G. To study Ly-4.2 alloantigen distribution on the surface of lymphoid cells as a method for determining B cells.

H. To analyze specific antigenic determinants p15 and gp70 on the type C virus envelope.

I. To detect natural antibody in primates' sera against gibbon and baboon type C viruses.

J. To study the relationship of fetal antigens to tumor-specific antigens using F_1 hybrid mice.

K. To locate carcino-embryonic antigen on human malignant cell surfaces.

L. To determine characteristics of a new type C virus isolated originally from transplanted SJL/J spontaneous reticulum cell neoplasm.

M. To examine a difference in tumor regression associated with the appearance of Gross leukemia virus pseudotypes in AKR and C58 mice.

Methods Employed:

A. through M. Various newly modified immunological methods -- i.e., humoral and cell-mediated micro-cytotoxicity tests, precipitation techniques using plastic templates, and immunoelectron microscopy in combination with cross-absorption and/or inhibition of antisera by various antigen sources -- have been fully used in mammalian systems.

Major Findings:

A. The presence of sarcoma cell surface antigen (SCSA) on three different cell lines [Kirsten sarcoma virus-transformed nonproductive BALB/3T3 (K-BALB)], and [normal rat embryonic kidney (NRK) transformed by Kirsten and woolly monkey sarcoma virus to nonproducer sarcoma cells (K-NRK and W-NRK)] has been examined by immunoelectron microscopy using rabbit anti-K-BALB, anti-K-NRK, and anti-W-NRK sera preabsorbed with normal embryonic rat cells, NRK cells, and BALB/3T3 cells. Four different specific SCSAs have been demonstrated: SCSAa, an antigen specific to the surface of W-NRK cells; SCSAb, a surface antigen common to K-NRK and W-NRK cells; SCSAc, a surface antigen common to K-BALB and W-NRK cells; and SCSAd, a surface antigen broadly reactive with the rabbit antisera to W-NRK, K-NRK, and K-BALB.

B. Pristane-pretreatment makes the mouse peritoneal cavity granulomatous. While pristane-pretreatment seems to be essential for the development of plasmacytomas, Abelson's virus enhances the development of plasmacytomas by 50%. Inoculation with Abelson's virus alone induced lymphosarcomas only, and pristance-pretreatment aided in the development of lymphosarcomas.

C. When the preparation of Streptococcus hemolyticus was injected intramuscularly into preleukemic mice twice a week, leukemia incidence was 50%, whereas a control group injected with phosphate buffered saline showed a 90% incidence in a coccus-treated group at the age of 11 months. In the plasma of mice at the preleukemic stage, the control group was positive for type C virus reverse transcriptase but the treated group was negative. Additionally, the former serum was negative for interferon but the latter was positive.

D. Some transplanted tumor lines and in vitro transformed cell lines during culture showed marked alteration of surface antigen expression. In particular, when transplanted tumor lines were cultured, they quickly changed surface antigen expression.

E. After the production of a certain endogenous type C virus from mouse cell lines, these cells inhibited ^{51}Cr-release from BALB/c Moloney sarcoma cells by the attacking lymphocytes of Moloney sarcoma-rejecting BALB/c mice. The inhibitory effect has been demonstrated only with cell lines releasing the endogenous type C virus. This surface antigen has been tentatively designated MEV-SA1 (mouse endogenous virus-associated surface antigen 1).

F. Peripheral blood platelets of quail and mice, when injected together with sarcoma viruses enhanced the growth of Rous sarcomas in chicken and Moloney sarcomas in BALB/c mice respectively. The effective factor appears to be contained in the supernatant of platelet homogenate after low-speed centrifugation.

G. Only 5.5% of the cells in the thymus carried the Ly-4.2 specificity, compared with 55.7% in the spleen and 20.9% in the lymph nodes,

H. The entire envelope of type C virus was strongly labeled with markers by immunoelectron microscopy using the antiserum against the whole virus. However, the labeling density of the viral envelope decreased when the antiserum against viral envelope p15 was used, and diminished further with the antiserum against gp70. The latter two antisera also reacted with the cell surface in areas smaller than the diameter of virions.

I. The sera of selected gibbon apes reacted with both the entire envelope of all type C viruses and small areas of cell surfaces of gibbon leukemia cells which were releasing abundant type C viruses. When these type C viruses were used to infect human rhabdomyosarcoma cells, one virus population reacted positively with the gibbon sera but another virus population did not react. Positive cell surface areas were smaller than the diameter of type C virus.

J. The antiserum against mouse embryo cells was found to contain many antibodies other than fetal antibody, e.g. autoantibody, antibody to normal thymocyte surface antigen, etc. The specificity of fetal antigen seems most likely to differ from tumor specific cell surface antigens.

K. Carcino-embryonic antigen was found located close to the plasma membrane of cultured human malignant cells (distance 15 ± 5nm).

L. An N-tropic type C virus isolated from a spontaneous reticulum cell neoplasm (Hodgkin's type) of an SJL/J mouse was propagated in NIH Swiss mouse embryo cell cultures. When the virus released from NIH cells was injected into BALB/c mice, 30% of mice developed the same type of neoplasm.

M. When Moloney murine sarcoma virus (MSV) was injected into AKR and C58 mice, 18% of sarcomas regressed, 55% progressed, and 27% persisted in AKR mice, whereas 98% of sarcomas grew progressively in C58 mice. By absorption and neutralization tests, AKR and C58 sarcoma cells were demonstrated to produce Gross leukemia virus pseudotypes. However, these sarcoma-bearing

mice were not suppressed immunologically.

Approximately 15% of this investigator's efforts have been spent in the administration of the Virus Cancer Program as a committee member of the Immunology Epidemiology Segment and a Project Officer.

Significance to Biomedical Research and the Program of the Institute:

A. The findings of SCSAa specific for murine sarcoma virus (MSV)-transformed cells is consistent with the known biologic and biochemical differences between woolly monkey sarcoma virus (WSV) and Kirsten-MSV. An antigen shared by W-NRK and K-NRK cells but not K-BALB cells (SCSAb) is likely to be due to a change of cell surface construction induced by each virus. Similarly, an antigen shared by K-BALB and W-NRK cells (SCSAc) also may be induced through indirect interaction of the viruses with the two host cell lines. Consequently, SCSAc existing on K-BALB and W-NRK cells may likewise be induced by the cooperation of WSV genome with a genetic function common to BALB/3T3 and NRK cells. The lack of cross-reactivity of K-NRK cells with SCSAc would argue that the effect of Kirsten-MSV on the rat cell must not produce this antigen in sufficient quantity to permit its detection.

B. Pristane-treatment and Abelson's type C virus-injection were essential for the induction of plasmacytomas and lymphosarcomas respectively. Combination of these treatments enhanced the development of both tumor types.

C. The lyophilized Streptococcus hemolyticus seems to have some component(s) which suppress type C virus replication, thus resulting in the reduction of and/or delay of leukemia incidence.

D. Mouse cells may contain several different type C viral genomes which are activated under certain circumstances. This phenomenon may reflect on the phenotypic expression of endogenous type C virus-associated surface antigens.

E. The Moloney pseudotype murine sarcoma virus source is usually contaminated with various type C viruses, including endogenous type C virus. After rejecting the Moloney sarcomas, BALB/c lymphocytes were sensitized by phenotypically expressed surface antigen (MEV-SA1) associated with certain endogenous type C viruses. Therefore, this antigen is not specific for Moloney sarcoma virus.

F. The role of peripheral blood platelets in virus-induced tumors was unclear. However, the results of this study clearly suggest the presence of enhancing factors of virus-induced tumor growth in platelets. It should be clarified by further studies whether the factor enhances malignant cell division or virus replication.

G. Ly-4.2 alloantigen on B-cells can serve as a marker for recognizing B-cells in Ly-4.2-positive mouse strains.

H. It is quite reasonable that the antigenic determinants of p15 and gp70 on type C viral envelope are distributed less densely than all classes of envelope antigenic determinants. Further studies are necessary to determine antigenic specificities by cross-direct-reaction and cross-absorption. Small positive areas on the cell surface may carry antigenic determinant(s) common to viral envelope antigens.

I. Human rhabdomyosarcoma cell-adapted gibbon type C viruses were classified into at least two populations, those reacting positively or negatively with gibbon natural antibodies. The results suggest three possibilities; a) gibbon type C virus changed its envelope antigenic specificity after adaptation to human cells, b) gibbon virus infection stimulated a type C virus in human rhabdomyosarcoma, or c) gibbon virus rescued rhabdomyosarcoma defective virus. Positive areas on the cell surface may contain antigen common to viral envelope antigen.

J. Since fetal antigen differed from tumor-specific antigen but the specificity of fetal antigen demonstrated on different tumors was common, fetal antigen seems most likely to be coded by certain viruses.

K. The finding that carcino-embryonic antigen is located a short distance from the plasma membrane differed from the report of Dr. Gold et al. that this antigen was located far from the plasma membrane in the fuzzy coat.

L. SJL/J spontaneous reticulum cell sarcoma (Hodgkin's type) virus may induce the same type of neoplasm in BALB/c mice but not in SJL/J. Alternatively, this virus may be modified by passage in NIH Swiss embryonic cells or switch on NIH cell endogenous type C virus which is capable of inducing Hodgkin's type neoplasms in BALB/c mice.

M. Naturally rescued sarcoma virus in AKR and C58 mice after inoculation with Moloney sarcoma virus contained Gross MuLV envelope as helper components. These two mouse strains showed different resistance to their sarcomas, suggesting the presence of differences in immunologic responses.

Proposed Course:

A. To analyze further the interaction of sarcoma virus and cellular genomes using human and guinea pig cells.

B. To study a possible mechanism of antigenic modulation by phagocytosis of type C virus or surface antigen with macrophages.

C. To purify the effective factor from Streptococcus hemolyticus and to combine it with specific immunotherapy of virus-induced tumors.

D. To apply this approach to human cell lines using platelet effective factors.

E. To clarify the relationship between the specificity of MEV-SA1 and humoral antibody from the same donor.

F. To isolate the effective factor from platelets, and to apply it to the detection of possible human type C virus.

G. Project completed; to be terminated.

H. To determine the specificity of individual antigenic determinant(s) at the molecular level and to expand the study of primate type C viruses.

I. To screen sera from healthy human donors as well as tumor patients using nonprimate type C viruses, after establishing the specificity of the viral envelope antigens. Simultaneously, to absorb gibbon natural antibody with different human tumors for finding possible human type C virus.

J. and K. To apply the results obtained in experimental systems to human tumors and placentas.

L. To determine the specificity of transplanted Hodgkin's type neoplasms established in BALB/c mice.

M. To investigate further the difference in immunologic responses to wild-type Gross MuLV in AKR and C58 mice.

Honors and Awards:

Presentation at Symposium on "Experimental Leukemias" of the XIth International Congress of Cancer Research in Florence, Italy, September, 1974.

Special lecture on "The Present and Future Status of Cancer Immunotherapy," in Chiba, Japan, March, 1975.

Chairman, Symposium on "Cancer Immunotherapy" of the 19th General Assembly of the Japanese Medical Association, Kyoto, Japan, April, 1975.

Nomination to the Editorial Board of the Journal of the National Cancer Institute.

Publications:

Aoki, T., Chieco-Bianchi, L., Plata, E.J., Sendo, F., Hollis, V.W. Jr., and Kudo, T.: Host immune response to virus-induced tumors: Some recent concepts. In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basal, Switzerland, S. Karger, 1974, pp. 23-36.

Aoki, T., Kvedar, J.P., Kudo, T., Plata, E.J., Sendo, F., and Hollis, V.W. Jr.: Enhancement of host immune response to cell surface antigens by the extract of Streptococcus hemolyticus. In Ito, Y. and Dutcher, R.M. (Eds.): Comparative Leukemia Research, 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 237-240.

Aoki, T.: A current status of tumor immunology. Infection, Inflammation and Immunity 4: 19-26, 1974 (in Japanese).

Greenburger, J.S., Stephenson, J.R., Aoki, T., and Aaronson, S.A.: Cell-surface antigens of murine sarcoma-virus-transformed nonproducer cells: Further evidence for lack of transplantation immunity. Int. J. Cancer 14: 145-152, 1974.

Aoki, T. and Hanafusa, H.: Cell surface antigens of type C RNA viruses as markers for detection of viruses. In Ito, Y. and Dutcher, R.M. (Eds.): Comparative Leukemia Research, 1973. Tokyo, Japan, University of Tokyo Press, 1974, pp. 441-442.

Herberman, R.B., Aoki, T., Nunn, M., Lavrin, D.H., Soares, N., Gazdar, A., Holder, H., and Chang, K.S.S.: Specificity of ^{51}Cr-release cytotoxicity by lymphocytes immune to murine sarcoma virus. J. Natl. Cancer Inst. 53: 1103-1111, 1974.

Aoki, T.: Reclassification and reconsideration of antigens on surfaces of tumors. Medical Chugai 27: 11-18, 1974 (in Japanese).

Aoki, T., Stephenson, J.R., Aaronson, S.A., and Hsu, K.C.: Surface antigens of mammalian sarcoma virus-transformed nonproducer cells. Proc. Natl. Acad. Sci. USA 71: 3445-3449, 1974.

Aoki, T., McKenzie, I.F.C., Sturm, M.M., and Liu, M.: Distribution of the alloantigen Ly-4.2 on murine B cells. Immunogenetics 1: 291-296, 1974.

Chieco-Bianchi, L., Colombatti, A., Collavo, D., Sendo, F., Aoki, T., and Fischinger, P.J.: Tumor induction by murine sarcoma virus in AKR and C58 mice: Reduction of tumor regression associated with appearance of Gross leukemia virus pseudotypes. J. Exp. Med. 140: 1162-1179, 1974.

Aoki, T.: A review on antigen of RNA oncogenic viruses. Medical Chugai 27: 311-318, 1974 (in Japanese).

Aoki, T.: Host immune response to tumors. Medical Chugai 27: 351-356, 1974 (in Japanese).

Aoki, T. and Sibal, L.: Immunology of virus-induced leukemias. In Japan Hematological Society (Eds.): Handbook of Hematology. Tokyo, Japan Maruzen, in press.

Aoki, T. and Sibal L.R.: Surface antigens associated with RNA type C viruses. Proceedings, XIth International Cancer Congress, Amsterdam, Excerpta Medica, in press.

Chang, K.S.S., Aoki, T., and Law, L.: Isolation of B-tropic type C virus from reticulum cell neoplasms induced in BALB/c mice by SJL/J type C virus. J. Natl. Cancer Inst., in press.

Aoki, T. and Sibal, L.R.: RNA oncogenic virus-associated antigens (and host immune response to them). In Becker, F.L. (Ed.): Cancer: A Comprehensive Treatise. New York, New York, Plenum Press, in press.

Sibal, L.R. and Aoki, T.: Immunological controls of RNA tumor viruses: antigenic characterization. Vapprosi Virusologif (Problems in Virology). Symposium on biology of oncogenic viruses of human and animal origin, Moscow, U.S.S.R. 1974, in press.

Aoki, T. and Chang. S.S.: Type C RNA viruses: Diverse immunological and etiological roles. In Raspe, G. and Bernhard, S. (Eds.): Advances in the Biosciences 12. Vieweg, West Germany, Pergamon Press, 1974, pp. 597-603.

Aoki, T.: Cell surface antigens of normal and neoplastic lymphohematopoietic cells. In Hersh, E. (Ed.): Immunological Aspects of Neoplasia. Baltimore, Maryland, Williams & Wilkinson, in press.

Kudo, T. and Aoki, T.: Specific antigens on the surface of tumor cells. In Kobayashi, H. and Tachibana, T. (eds.): Tumor Immunology. Tokyo, Japan, Asakura Publishing Company, 1974, pp. 37-45 (in Japanese).

Serial No. ZO1-CP-04891-03-VLL

1. Viral Leukemia and Lymphoma Branch
   OADVO, Division of Cancer Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the biology of DNA and RNA oncogenic viruses

Previous Serial Number: Same

Principal Investigator: Dr. Herbert K. Oie

Other Investigators: Dr. Dharam V. Ablashi
Dr. Adi F. Gazdar
Dr. Shigeko Nomura

Cooperating Units:

Inside NIH
Dr. Samuel Baron, LBV, NIAID
Dr. Josephine Simonds, VB, NCI

Outside NIH
None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To determine the biologic properties of xenotropic murine type C viruses.

2. To investigate the in vitro transforming potential and other properties of Herpesvirus saimiri.

3. To determine the biologic properties of viruses isolated from rat mammary tumors.

Methods Employed:

1. Murine type C virus production was determined by the complement fixation test or the RNA dependent DNA polymerase (RDDP) assay. Sarcoma virus pseudotypes of murine leukemia viruses were assayed by focus formation in mouse or rat cells. All cells were cultured by routine tissue culture techniques.

2. Infectious virus was assayed by a plaque assay using a "soft" overlay consisting of Eagle's minimal essential medium, 5% fetal calf serum, and 0.3% agarose.

3. Virus production was followed by the RDDP assay. Tumorigenicity of cells was tested in newborn or adult Sprague-Dawley rats. Standard techniques were used for the preparation of tumor tissues and cells for microscopical examinations.

Major Findings:

1. Information on the host range properties of murine endogenous type C viruses has generally been limited. A study of the susceptibilities of a variety of animal cells to both ecotropic and xenotropic viruses showed that the ecotropic viruses tested infected only murine and rat cells, whereas the X-tropic viruses replicated in cells from a large number of mammalian species. While rat cells were susceptible to both types of viruses, hamster cells proved to be uniformly resistant. The X-tropic viruses crossed class barriers to infect cells from three avian species, but did not replicate in insect, fish or reptile cells. Generally, a great variability in the susceptibilities to X-tropic viruses existed among cells from heterologous species as well as among cells from a single species. A carrier culture of a spontaneously transformed rhesus monkey cell line infected with a human cell-adapted Rauscher leukemia virus has been maintained for more than two years. The virus produced by this carrier culture, labeled primate Rauscher leukemia virus (PRLV), infected cells from many mammalian species, but not BALB/c (B-type) or NIH/3T3 (N-type) murine cells, indicating that it may be an X-tropic virus. PRLV did replicate in a cell line, SC-1, derived from a wild mouse. However, even after 20 passages of the PRLV-infected SC-1 cells, the progeny virus retained the host range of PRLV and did not infect N-type or B-type murine cells. PRLV was shown to be a murine virus by the complement fixation test. BrdU treatment of the carrier culture caused an increase in virus production per cell reaching a peak 4 days after BrdU treatment. Marmoset and rhesus monkeys infected with a cell-free concentrate of PRLV have remained disease-free for over nine months with little or no detectable antibody response.

2. A spontaneously transformed rhesus monkey cell line chronically infected with H. saimiri (HVS-MEST) has been adapted to grow at 32°C or 37°C

with continued production of cytopathic changes, viral antigens, and infectious virus. A time course study of virus production at 37°C showed that maximal infectious virus production occurred 8-10 days after subculturing HVS-MEST. Unlike findings by other investigators, more virus was released by HVS-MEST than remained cell-associated. In addition, more virus was produced when HVS-MEST was incubated at 37°C than at 32°C. In contrast, owl monkey cells infected with H. saimiri produced optimal yields when incubated at 32°C. Using 8% polyethylene glycol 6000 in the presence of 0.5M NaCl, partial purification and concentration (10-20-fold) of infectious virus were achieved. When small volumes (10-50 ml) of crude virus were concentrated, 90%-95% recovery of infectious virus was obtained, but when larger volumes (> 1000 ml) were used, the efficiency of recovery was reduced to about 50%.

3. Of the many spindle-shaped and epithelioid cell clones isolated from three mammary tumors of an in vivo maintained rat tumor line, an epithelioid cell clone was selected for study. This cell clone was positive for rat type C virus, gs antigen, and RDDP activity. Inoculation of cells into newborn or adult Sprague-Dawley rats resulted in the production of tumors within 10-14 days. Histologically, these tumors were typically differentiated adenocarcinomas. Normal rat kidney cells infected with a concentrated cell-free virus preparation neither transformed nor produced progeny virus. In aged cultures, these cells developed "dome" structures which characterize the in vitro growth of epithelioid cells isolated from human and murine mammary tumors.

Significance to Biomedical Research and the Program of the Institute:

Neoplastic diseases similar to those found in man afflict other animals. Comprehensive studies of these animal neoplasia and their causative agents, both in vitro and in vivo, could provide invaluable information toward the solving of human cancer. These animal and cell culture model systems could also be useful for the testing of potential anti-cancer drugs and new methods of therapy.

Proposed Course:

1. The growth of ecotropic and xenotropic viruses in cells of different species will be studied further, with particular interest in the effect(s) that long term cultivation in heterologous cells might have on the host range of these viruses. Mechanism(s) controlling resistance and permissiveness by different cells to these two types of viruses will also be investigated. Efforts to resolve the relatedness of PRLV to ecotropic and xenotropic viruses will be pursued using immunological and biochemical techniques. The ongoing in vivo studies will be continued.

2. Further studies on the biology of H. saimiri will be pursued. Mutants with reduced capacity for cytopathology or growth at low temperatures

(temperature sensitivity) will be isolated and tested for cell transforming potential, oncogenicity, and possible usage as vaccines. In vivo studies will be continued.

3. The epithelioid cell clone will be further characterized for possible mammary origin by testing for hormonal effects on cell growth and casein production. The biology of the type C virus produced by these cells will also be studied.

Honors and Awards:

None

Publications:

Didier, M.L., Ablashi, D.V., Oie, H.K., Armstrong, G.R., Chu, E., and Rabson, A.: Some biological properties of Herpesvirus saimiri (HVS) from chronically infected monolayer and suspension cultures. Monograph, 2nd International Symposium on Oncogenesis and Herpesviruses. Nuremberg, Germany (Oct. 14-16), 1974, in press.

SUMMARY REPORT

C. 1. BREAST CANCER VIRUS SEGMENT

July 1, 1974 through June 30, 1975

The Breast Cancer Virus Segment activities involve three major areas of investigation: (I) The study of the structure, and control of expression, of the Mouse Mammary Tumor Viruses (MMTVs), (II) the characterization of Mason-Pfizer Monkey Virus (MPMV) and a type B virus isolated from guinea pigs, and (III) the further characterization of the particulates isolated from human milk and breast tumors, and the particle being released from the MCF-7 human breast tumor cell line. The virus-related nucleic acid information observed in human breast tumors is also being further characterized.

Treatment of MMTV with nonionic detergent resulted in the isolation of a 1.24 g/cc core-like particle which had high molecular weight RNA, reverse transcriptase, and MuMTV protein. By electron microscopy, these particles morphologically resemble the subviral cores observed in other oncornavirus preparations. The 1.24 particles lacked immunological surface components but contain p28 activity. PAGE studies are in progress, but initial results indicate that these particles are enriched for p28 and p14.

Major efforts using tissue culture were devoted to the production of sufficient quantities of MuMTV for virus purification and production of specific antibodies.

A quite interesting result was the induction in vitro of MuMTV by dexamethasone from cells of the low mammary cancer mouse strains C57BL and BALB/c. MuMTV is induced in vivo in these strains with difficulty. These data suggest that possibly all strains of mice carry MuMTV and that humoral and other factors control the release of type B endogenous virus in these strains.

Radioactively labeled 60-70S RNA of various MMTVs (GR, RIII, Balb/cfC3H) were obtained from cell cultures of mammary tumors and shown to be free of contaminating murine leukemia viruses by a variety of techniques. These RNA's were used, in competition hybridization experiments, to determine the relationship of the genomes of various MMTVs to one another. Direct molecular hybridization of these ^{3}H 60-70S RNAs to the DNA of organs of several mouse strains, was used to determine the copy number and distribution of MMTV proviral DNA. The proteins of MMTV from various mouse strains were also compared.

Infection and replication of MuMTV was obtained when the CRFK-F2 cat kidney cell line was exposed to Ficoll density gradient purified RIII virus. No morphological difference was observed in the infected cells. Typical type B particles were recovered from supernatant fluids from cat kidney cultures serially passaged over 12 months. Virus production was also confirmed by

membrane immunofluorescence. The infected cells have been certified to be from cat origin by a variety of standard tests and the virus produced is infectious and tumorgenic in mice.

An investigation was initiated to coordinate a study of mammary gland chromatin biochemistry, emphasing the role of non-histone chromosomal proteins with an examination of the transcription of unique sequence DNA, viral genes, and specific messenger RNA. After approximately six months of work, investigators have been successful in the isolation of casein-rich message and the reverse transcription of the casein message to provide a cDNA from rat casein. This material is being studied for the effects of hormones in in vitro systems of the rat mammary gland. The second phase of the study is to prepare similar materials from the mouse mammary glands. Towards this end, these investigators have been supplied with up to 100 gms of mammary gland and large quantities of mouse casein for their studies. These efforts will provide information relative to the hormonal regulation of viral with other integrated DNA sequences at the level of DNA to RNA transcription.

Linkage experiments are well underway for GR-MTV (exogenous). Testing has been completed for over twenty markers. Genetical analysis to determine the number of genes which control induction of C3Hf-MTV (NIV-Endogenous) and whether NIV induction is linked to GR-MTV induction has begun. Important findings were that the extremely high expression of MTV-gs antigen in the genital tract organs of GR mice might offer an explanation for the fact that BALB/c females, if caged with GR males become positive for MTV-gs antigen in their milk. Another finding was that young adult male mice are suitable to study the induction gene of MTV since they can be selected according to whether they are expressing MTV-gs antigens in extracts of the coagulating gland and the epididymis. Investigations with congenic lines on the C57BL/10 ScSn background have shown that the incidence of mammary tumors after administration of C3H-MTV is dependent on the histocompatibility locus H-2. Resistant strains are those with the haplotype $H\text{-}2^b$ and $H\text{-}2^g$. Probably genes in or near the D-end of the H-2 locus determine the susceptibility of MTV. It has been also shown that the $H\text{-}2^g$ allele determines resistence to MTV also on the 020-background. The incidence of mammary tumors in the C57BL strain is dependent on the kind of hormonal stimulation of the mammary gland. Force breeding is insufficient to induce mammary tumors in the absence of MTV. Continuous overdose of prolactin by way of hypophyseal isograft in the kidney induces a high incidence of mammary tumors even in the absence of MMTV.

Studies concerning the characterization of the MMTV-induced cell surface antigens were begun to aid in the understanding of gene transmission and gene action. Currently a transplanted GR leukemia of thymic origin which shares the MLr, Thy. 1.2, and H-2.8 antigens with mammary tumors is being used for these studies. The MLr antigen is also present in B-particle envelope and is a glycoprotein of 52,000 daltons. The methodology for accurate and rapid detection of these antigens on various test cells was worked out.

Successful suppression of MuMTV expression and tumorgenesis by immunization with MuMTV has been accomplished. Af/C57BL and RIIIf/C57BL were immunized

with killed MuMTV in complete Freund's adjuvant. Expression of MuMTV up to the 10th lactation was significantly suppressed and tumor incidence at 15 months of age was reduced. Milks from high-mammary tumor mouse strains A, BALB/cf C3H, RIII and C3H and milk from low tumor RIIIf (late lactations) were titrated for infectivity in C57BL, BALB/c, Af, and RIIIf mouse strains. There was a wide variation in susceptibility of different mouse strains to the same virus, e.g. RIIIf milk was highly infectious in BALB/c and Af but not in C57BL.

New studies have been initiated to test whether it is possible to immunize mice against mouse mammary tumor virus (MMTV) without exposing them, in the process, to structurally intact virus. The contractor will investigate whether immunization of mice with individual, purified structural proteins from MMTV can decrease or abolish their susceptibility to spontaneous as well as virus-induced mammary tumors. Isolation and purification of the structural proteins will be performed under the mildest possible conditions thus ensuring conservation of the native conformation of the proteins. Immunization procedures are designed in such a way as to (a) increase as much as possible the immunogenicity of the proteins, of which, only very small doses are to be injected; (b) stimulate a cell mediated, rather than humoral, immune response.

MPMV was grown in four different cell lines (CMMT, Rhesus foreskin, AV3 and Human NC-37) and the proteins of purified virus analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). The glycoproteins of MPMV, grown in different cell lines, were also analyzed via labeling of cultures with radioactive glucosamine and compared to those of MMTV and MuLV. The size and subunit structure of the RNA of MPMV was studied and compared to Rauscher murine leukemia virus RNA. Dissociation of 60-70S RNA by heat or formamide resulted in conversion to identical subunit stuructures of 2.8×10^6 daltons for both MPMV and MuLV RNA. Treatment with lower amounts of formamide revealed a partial dissociation to intermediate RNA structures. Complete heat dissociation of MPMV 65S RNA also released three low molecular weight RNA species.

KC cells formed massive syncytia when co-cultivated with MPMV infected cells. This phenomenon can be used as the basis of a rapid end-point assay for MPMV and related viruses such as the AO and J-96 viruses recently isolated from human cell lines. Efforts are continuing to refine this assay system further so that enumeration assays, comparable to the XC test for mouse leukemia virus, can be developed. A radioimmunoassay for the purified major core protein, p27, of MPMV was developed and has been used to demonstrate the antigenic relatedness to MPMV of both monkey and human viral isolates. Additional radioimmunoassays for other MPMV associated polypeptides are being developed. In addition, a new low molecular weight MPMV-associated polypeptide has been detected and is currently being characterized. Human tumors are being screened for antigens related to MPMV p27 by the radioimmunoassay described above. MPMV induces soft agar colony formation by monkey foreskin (FS-2) cells. The properties of cells isolated from these colonies, in particular their ability to induce tumors in vivo, are being investigated. In the initial study, tumors were induced in nude mice following inoculation of these

cells. The tumors were grown in vitro, and the resulting cell line was shown to contain monkey cells. Cloned stocks of MPMV, some of which have already been isolated, are being studied in order to examine the biological interactions, if any, that occur between MPMV and SSV-1 related C type viruses.

Studies are continuing to determine if MPMV inoculated monkeys were indeed infected with MPMV. Eight monkeys were sacrificed and tissues from various organs were assayed for: (a) infectious virus by the KC test; (b) MPMV antigen by immunodiffusion; (c) viral specific DNA by molecular hybridization. No infectious virus or antigen were detected, but MPMV-specific DNA was found in the DNA of several organs of several infected monkeys.

Radioactive DNA (^{3}H-cDNA) complementary to the RNA of Mason-Pfizer Monkey Virus was used in molecular hybridization experiments to demonstrate sequence homology between MPMV RNA and RNA of human malignant breast tumors. Hybridization products were analyzed by cesium sulfate equilibrium density centrifugation and hydroxylapatite chromatography. Twenty-seven or thirty-nine malignant breast tumors tested contained MPMV-related RNA while no sequence homology was found in RNA from human benign breast tumors or normal breast, spleen, liver or kidney. Hybridizations between MPMV ^{3}H-DNA and human breast adenocarcinoma RNA showed a $C_rt_{1/2}$ of approximately 2×10^4. Thermal analysis of the hybrids demonstrated a T_m of approximately 80°C. Hybridization of radioactively labeled MPMV 60-70S RNA of breast tumor DNA is in progress.

Particulates with the properties of cores and/or ribonucleoproteins of RNA tumor viruses have been isolated from Sterox-SL-treated fractions of murine and human mammary adenocarcinomas. These particulates have an RNA-directed DNA polymerase, a 60-70S RNA, and a density of 1.26 g/ml or greater in sucrose equilibrium density gradients. Their uniquely higher densities lead to banding in regions comparatively free of cellular contaminants. These circumstances minimize some of the technical complications of performing the simultaneous detection assay in the presence of cell debris. Further purification and characterization of the reverse transcriptase from human malignant breast tumors, human milk, and human nipple discharge is now in progress. To date, 31% (9/29) malignant and 25% (2/8) non-malignant breast tissues contained RNA with significant homology to MuMTV RNA. Only about 5% base pair mismatching was detected in the MuMTV RNA-human breast tumor RNA hybrids.

Studies are currently in progress to characterize the particle being released by the human breast tumor cell line MCF-7. Preparation of hyperimmune monospecific and polyvalent antiserum to MuMTV has permitted close scrutiny of the relatedness of 734B and MuMTV. Comparison by immunofluorescence of 734B producer clones of MCF-7 cells with non-producer clones, human epithelium cells, and BALB/c cells from MTV-free mammary glands or BALB/c cells infected with MuLV, showed no reactivity with the control cells and 5-7% reactive cells in the 734B producer cultures. The positive cells showed granular fluorescence in the cytoplasm. Hybridization of ^{3}H-DNA copies of the viral genome of 734B to human DNA and to DNA of tissues from animals that might have been sources of viral contamination was studied. Purified radioactive DNA

probe was purified and then hybridized with DNA from human placenta, mouse liver, bovine thymus, cat liver, monkey liver, and hamster liver. Bacterial DNA was also hybridized with viral DNA under identical conditions to measure non-specific binding. Using the most stringent criteria, the DNA of the 734B virus particle showed 28-35% double-strandedness complementarity with human placental nuclear DNA. Average values of 5% were obtained with the control tissues. Further studies to characterize the MCF-7 particles are in progress.

Dr. Jeffrey Schlom, OAD, DCCP, Chairman
Dr. Wade Parks, VC, DCCP, Vice Chairman
Dr. Ernest J. Plata, VLL, DCCP, Executive Secretary

BAYLOR COLLEGE OF MEDICINE (NO1-CP-43385)

Title: Regulation of Gene Expression in Mouse Mammary Cancer

Contractor's Project Director: Dr. Susan H. Socher

Project Officer (NCI): Dr. Wade Parks

Objectives: To examine the regulation of gene expression in mouse mammary tissue and characterization of chromatin from normal mammary tissue of virgin, mid-pregnant and lactating mice, pre-neoplastic alveolar nodules and mammary tumors in high and low MMTV expressor mice. To study the mechanisms of modulation of DNA to RNA transcription in mammary cells using RNA transcribed *in vitro* from casein mRNA and viral sequences as probes.

Major Findings:

1. Isolation and characterization of mouse tissue chromatin. Chromatin was isolated from nuclei from whole tissues from pregnant and virgin mammary glands and some mammary tumors analyzed for its mass composition, template activity and associated proteins (histone and non-histone). Low levels of template activity were observed in inactive glands as well as in glands isolated early in pregnancy. An increase in template activity was observed in mid-pregnancy and this level was maintained or slightly increased during the remainder of pregnancy and through lactation. No demonstrable changes in histone species were detected and minor changes were found in non-histone protein species associated with the chromatin. This would suggest that the level of control gene expression in mammary tissues occurs at a molecular level. Most mammary tumors show levels of template activity similar to or lower than that observed during lactation and similar histone and non-histone protein profiles were observed in both tissues.

2. Using model system rat casein messenger RNA (mRNA), rat casein mRNA has been purified by sequential steps involving chromatography on Sepharose 4B, purification on dt-cellulose chromatography and sucrose gradient centrifugation after heat denaturation. The mRNA activity of peak fractions composed of 70% of the casein mRNA and final purification represented 63 fold purification compared to the starting RNA extract. It should now be possible to generate specific probes of [^{3}H]-labelled complementary DNA (cDNA) for hybridization analysis in the rat mammary gland system.

3. The techniques that have worked for the purification of rat mammary casein mRNA are being applied to mouse casein mRNA with similar results.

4. Antisera have been prepared against the purified rat casein and shown to react specifically by immunodiffusion. A specific antibody against band 3 casein shows little or no cross-reactivity with band 1 casein when analyzed immunoelectrophoresis.

Significance to Biomedical Research and the Program of the Institute: Considerable evidence suggests that the control of the inherited mouse mammary tumor virus occurs at the level of DNA to RNA transcription. Studies designed to facilitate an understanding of the regulation of this control will lead to an increased understanding of the effects of hormones in differentiation on the regulation of viral and non-viral genes involved in carcinogenesis.

Proposed Course: To continue the purification of mouse casein mRNA. To reverse transcribe both mouse and rat casein mRNA's and to develop a specific radioimmunoassay for the measurement of casein polypeptides.

Date Contract Initiated: 26 June 1974

Current Annual Level: $74,856.00

UNIVERSITY OF CALIFORNIA, DAVIS (NO1-CP-33253)

Title: In Vitro Cultivation of Human and Mouse Mammary Tumor Virus

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. Robert Bassin

Objectives: This contract seeks to apply to the human breast tumor culture system the various experimental conditions developed in the mouse mammary tumor model. These efforts are directed towards the possible isolation and identification of a human mammary tumor virus. Work continues on the characterization of MuMTV and definitions of viral synthesis and production.

Major Findings: A 14S component has been isolated from the envelope of MuMTV which contains all of the MuMTV glycoproteins but lacks p28 and other nonglycositated proteins. This 14S component has many of the properties predicted for MuMTV "spikes" but has not as yet been completely characterized.

Treatment of MuMTV with nonionic detergent has resulted in the isolation of a 1.24 g/cc core-like particle which has high molecular weight RNA, reverse transcriptase, and protein. By electron microscopy, these particles morphologically resemble the subviral cores observed in other oncornavirus preparations. The 1.24 particles lack immunological surface components but contain p28 activity. PAGE studies are still in progress, but initial results indicate that these particles are enriched for p28 and p14.

In the process of developing a DNA probe for MuMTV, it was observed that the apparent production of cDNA ceases after the first three hours in reverse transcriptase reaction mixtures. A subsequent series of experiments has

indicated that the MuMTV reverse transcriptase is thermolabile. Preincubation in 37°C, for example, reduces the endogenous reaction by a factor of 2 and the exogenous reaction by a factor of 10. A more detailed study of the thermolabile of MuMTV reverse transcriptase is in progress.

Data concerning human immunoglobulins which bind to MuMTV are being collected. Current data do not differ from those previously reported - the binding appears to be a nonspecific reaction.

A project dealing with solid phase RIA has been initiated. Initial experiments indicate that the binding of protein to plastic surfaces is easily reversible, a fact which has hindered both quantitation and reproducibility.

Major tissue culture efforts have been devoted to the accumulation of sufficient quantities of MuMTV for virus purification and production of specific antibodies. Twenty-five mg have been accumulated during the past two months.

Separation of MuMTV-associated proteins on guanidine·HCl columns has been disappointing. Although three major and two minor peaks can be resolved, clear isolation of individual proteins such as can be obtained with other oncornaviruses has not been achieved. In exchange, chromatography has been substituted as a possibly more applicable techniques for MuMTV.

A number of MTV-derived proteins have been inoculated into rabbits for the production of antibodies.

Nucleic acid hybridization technology and the hyperplastic alveolar outgrowth (HOG) transplantation program have been instituted and are being put to use in this program.

Comparison of MuMTV produced in vitro by primary cultures and established cell lines has been of continuing interest, and collaborative programs with other investigators in the Breast Cancer Virus Segment are continuing.

Significance to Biomedical Research and the Program of the Institute: The knowledge and technology gained on the in vitro cultivation and structure of MMTV may be directly applicable to a human breast cancer virus.

Proposed Course: The activities described will be continued at the current contract level.

Date Contract Initiated: 01 February 1972

Current Annual Level: $121,551

HOWARD UNIVERSITY COLLEGE OF MEDICINE (NO1 CP 43287)

Title: Correlation of Molecular Virology Studies to Diagnosis of Breast Cancer

Contractor's Project Director: Dr. William Funderburk

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: This contract is aimed at determining if there is a correlation between the presence of reverse transcriptase activity in human spontaneous nipple discharge, breast duct fluids, or cyst fluids and clinical evaluation of patients by: (a) palpation, (b) xerography of both breast, (c) pap smears by conventional and millipore techniques, and (d) contrast mammography on patients with ductal discharges.

Major Findings: Approximately 170 patients have entered this study thus far. The methodology for the biochemical examination of these fluids, which are produced in very small quantities, has been generally worked out. The clinical procedures have included 77 duct aspirations, 63 Needle aspirations, 117 Papanicolau smears (72 conventional and 45 millipore), 63 Xerograms, and 23 Contrast mammograms. Pools of breast fluids have thus far been examined biochemically in collaboration with Meloy Laboratories, Springfield, Virginia (Contract No. NO1 CP 43223). These include spontaneous nipple discharge, breast duct fluids, and cyst fluids. Several of these fluids have been positive in endogenous DNA polymerase reactions. Analysis of reaction products by cesium sulfate equilibrium centrifugation revealed acid precipitable ^{3}H-TTP in the RNA and DNA:RNA hybrid regions of gradients. Heating at 68°C for 10 minutes in the presence of formamide showed a shift of approximately 90% of these cpm to the DNA region of the gradient, indicating hydrogen bonding of de novo synthesized ^{3}H-DNA in an endogenous RNA. Numerous fluid samples have also been positive using the template-primer poly rC:oligo dG$_{(10-12)}$ or poly rCm:oligo dG$_{(10-12)}$. Both have previously been shown to be rather specific for viral reverse transcriptase. Experiments to rule out possible contamination by terminal transferase or novel cellular DNA polymerase activities are in progress. The methodology is now developed to begin testing of individual specimens.

Significance to Biomedical Research and the Program of the Institute: These studies are important because they may yield some fundamental information that will contribute directly to the NCI objectives of better diagnostic methodology for breast cancer. The search for an etiologic agent of human breast cancer requires that this spontaneous fluids be examined, since they could be the most ideal source of a possible human breast cancer virus.

Proposed Course: The contractor intends to continue the characterization of the suspected reverse transcriptase in breast fluids, to optimize assay conditions for sensitivity and specificity, and to determine if there is a correlation of this activity with the clinical tests and diagnosis included in the screening battery.

Date Contract Initiated: 01 March 1974

Current Annual Level: $29,483

INSTITUTE FOR MEDICAL RESEARCH (NO1-CP-33339)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. Ernest J. Plata

Objectives:

1. To characterize the mammary tumor system of mice as a model for human breast cancer.

2. To resolve whether there is any relationship between murine mammary tumor virus (MuMTV) and human cancer.

3. To determine the significance of virus-like particles resembling oncogenic RNA viruses present in some human milks and breast tumors.

Major Findings: Successful, repeated infection of a cat kidney cell line with MuMTV. Infection and replication of MuMTV was obtained when the CRFK-F2 cat kidney cell line was exposed to Ficoll density gradient purified RIII virus. No morphological difference was observed in the infected cells. Typical type B particles were recovered from supernatant fluids from cat kidney cultures serially passaged over 12 months. Virus production was also confirmed by membrane immunofluorescence. The infected cells have been certified to be from cat origin by a variety of standard tests and the virus produced is infectious and tumorgenic in mice.

Establishment of a mouse cell line that produces large quantities of MuMTV. A continuous cell line derived from a BALB/cf C3H mammary tumor is continuously releasing MuMTV into the supernatant culture fluids (1×10^8 particles/ml). The virus produced is infectious in BALB/c and C57BL mice.

Confirmed findings of different susceptibility of various strains of mice to MuMTV derived from different host strains. Milks from high-mammary tumor mouse strains A, BALB/cf C3H, RIII and C3H and milk from low tumor RIIIf (late lactations) were titrated for infectivity in C57BL, BALB/c, Af, and RIIIf mouse strains. There was a wide variation in susceptibility of different mouse strains to the same virus, e.g. RIIIf milk was highly infectious in BALB/c and Af but not in C57BL.

Characterization of the morphology and chemical composition of MuMTV. The morphology of MuMTV was studied by the newer techniques of electron microscopy; freeze-etching, critical point drying, and modifications in fixation and preparative procedures. Under most conditions, MuMTV gave a

biomodal size distribution. The protein and glycoprotein composition of MuMTV was studied by using techniques of metabolic or extrinsic radioactive labeling followed by dissociation by a variety of means including organic solvents. The disrupted labeled components were separated by column chromatography and fractions identified as p23, p27, p28, p25, gp34, gp52, gp55, gp60, gp68, gp78 and gp90 were isolated. The two major polypeptides p55 and p28 were identified as GS1 and possibly GS2, respectively. Comparison of the composition of MuMTV from different strains was begun.

Successful suppression of MuMTV expression and tumorigenesis by immunization with MuMTV. Af/C57BL and RIIIf/C57BL were immunized with killed MuMTV in complete Freund's adjuvant. Expression of MuMTV up to the 10th lactation was significantly suppressed and tumor incidence at 15 months of age was reduced.

Reverse transcriptase from human milk particles was partially characterized and compared with that of MuMTV. The human enzyme had a molecular weight of approximately 100,000 daltons. The murine and human enzymes differed significantly in the stability, template preferences, and utilization of nucleotides. The same criteria indicated that there may be at least two distinct reverse transcriptase activities in the human milk particulates studied.

Detection of MuMTV-related RNA in human breast tumors. To date, 31% (9/29) malignant and 25% (2/8) non-malignant breast tissues contained RNA with significant homology to MuMTV RNA. These RNA sequences hybridized between 18 to 77 percent of the MuMTV DNA probes at C_rt values greater than 10^4. The C_rt curve analysis of these positive tumor RNAs showed very low amounts of MuMTV related RNA ranging from 1 to 8 molecules per cell. Only about 5% mismatching was observed in these MuMTV DNA-human RNA hybrids judging from the differences in Tm.

Detection of an antigen in human milk cross-reactive with MuMTV. Concentrated human milk virus-like particles were disrupted with detergent and salt, and fractionated by ion exchange chromatography. Preliminary assays have demonstrated a MuMTV-related antigen which was detectable by gel immunodiffusion and also was positive for reverse transcriptase activity after ion exchange chromatography and further concentration.

Significance to Biomedical Research and the Program of the Institute: The current results published by this laboratory are of great potential significance to the research programs of the NCI. The demonstration of *in vitro* infectivity by MuMTV and productive infection of cells of another species different from the species of origin opens up new areas of study. This importance is supported by the demonstration of MuMTV related antigens and RNA in certain human breast tumors and human milk. The resolution of whether a relationship exists between human breast cancer and a viral agent sharing genetic and antigenic information with MuMTV is of the highest priority. The immune protection against mammary tumors of mice vaccinated with inactivated MuMTV is also a significant advance. Thus continued work

in this area is consistent with the objectives of the program of the NCI especially concerning developments in improved diagnosis, treatment, and possible prevention of human cancer.

Proposed Course: The potentially significant findings reported will be confirmed and extended. It is of great importance that additional data be generated to support the observations of possible hybridization of MuMTV probes with RNA of human breast tumors. The immunological studies also warrant consolidation. It is expected that the next period will be scientifically productive and may lead subsequently to possible applications of proven techniques to human systems.

Date Contract Initiated: 28 June 1968

Current Annual Level: $460,000

MASON RESEARCH INSTITUTE (NO1-CP-33358)

Title: Study on the Role of Hormonal Factors on Induction of Breast Tumors

Contractor's Project Director: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: The principal aim of this contract is to define whether the Mason-Pfizer Mammary Virus (MPMV) is an oncogenic virus in monkeys. The contractor is monitoring approximately 100 monkeys inoculated with MPMV to determine: (a) development of tumors, (b) development of immunity to MPMV, and (c) appearance of MPMV antigens and nucleic acids in tissues of normal and inoculated monkeys.

Major Findings: There are presently a total of 23 female rhesus, 2 male rhesus, and 3 female cynomologous monkeys that have been inoculated neonatally with MPMV and being held under observation until maturing to the age of 18 months before being treated with hormones. A total of 30 adult rhesus females are under continuous steroid therapy; 20 have been inoculated with MPMV directly into the mammae and 10 are serving as controls. Lactation has been induced in all animals and milk samples have been collected and freeze-preserved. Attempts to detect evidence of virus by electron-microscopy, immunological tests for virus antigen, and reverse transcriptase activity are now in progress in collaboration with other VCP contractors and NCI scientists.

Lactation and uterine hypertrophy were induced in 5 non-pregnant female rhesus monkeys by exogenous 17β estradiol and progesterone. Hypertrophic uteri were then removed surgically. Following hysterectomy there was a significant increase in milk secretion and in serum levels of estrone and estradiol. The increased mammary secretary activity was correlated with the increased levels of circulating estrogen following hysterectomy indicating a significant competition between the uterus and mammae for estrogen. It would appear that hysterectomy may favor stimulation of

steroid responsive neoplastic mammary tissue, and be contraindicated following ovariectomy as a therapeutic procedure.

In a normal rhesus female, received from Bionetics Research Laboratory, lymphadenopahty and lymphocytosis became evident one week after MPMV injection and persisted for approximately 5 weeks. MMPV antigen was detected in lymph nodes by fluorescent antibodies and electron microscopy.

Studies were conducted in collaboration with other VCP laboratories to determine if MPMV inoculated monkeys were indeed infected with MPMV. Eight monkeys were sacrificed and tissues from various organs were assayed for: (a) infectious virus by the KC test, (b) MPMV antigen by immunodiffusion, (c) viral specific DNA by molecular hybridization. No infectious virus or antigen were detected, but MPMV-specific DNA was found in the DNA of several organs of several infected monkeys.

A radioimmune assay has been set up by the contractor to detect MPMV p27 protein. Serum from infected and control monkeys are being tested to detect the presence of MPMV antibodies or antigen.

Significance to Biomedical Research and the Program of the Institute: This project is important to the objectives of the NCI because currently it represents the only available primate model for a viral induced cancer. The proof of oncogenicity for this virus is essential.

Proposed Course: To continue the studies described to collect the data necessary to serve as basis for decisions concerning continued long-term holding and observation of these animals.

Date Contract Initiated: 09 June 1970

Current Annual Level: $160,615

MELOY LABORATORIES, INC. (NO1-CP-43223)

Title: Molecular Studies of Human and Animal Cancer with Emphasis on Breast Carcinoma

Contractor's Project Director: Dr. John Verna

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: This program is aimed at the use of techniques in molecular biology to determine a viral involvement in animal and human mammary carcinoma. The program is divided into several major areas of interest: (a) molecular studies of the mouse mammary tumor viruses (MMTV), (b) molecular studies of Mason-Pfizer Monkey Virus (MPMV) and related isolates, and (c) biochemical evidence of an oncornavirus in human milk and human breast tumors.

Major Findings: Radioactively labeled 60-70S RNA of various MMTV (GR, RIII, Balb/cfC$_3$H) were obtained from cell cultures of mammary tumors and shown to be free of contaminating murine leukemia viruses by a variety of techniques. These RNA's were used in competition hybridization experiments to determine the relationship of the genomes of various MMTVs to one another. Direct molecular hybridization experiments of these ^{3}H 60-70S RNAs, to the DNAs of various organs of several mouse strains, was used to determine the copy number of distribution of MMTV proviral DNA. The proteins of MMTV from various mouse strains have also been compared.

The protein constituents of MPMV were also studied. MPMV was grown in four different cell lines (CMMT, Rhesus foreskin, AV3 and human NC-37) and the proteins of purified virus analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). The glycoproteins of MPMV, grown in different cell lines, were also analyzed via labeling of cultures with radioactive glucosamine and compared to those of MMTV and MuLV.

The size and subunit structure of the RNA of Mason-Pfizer Monkey Virus (MPMV) was studied and compared to Rauscher murine leukemia virus (MuLV) RNA. Dissociation of 60-70S RNA by heat or formamide resulted in conversion to identical subunit structures of 2.8 x 10^6 daltons for both MPMV and MuLV RNA. Treatment with lower amounts of formamide revealed a partial dissociation to intermediate RNA structures. Complete heat dissociation of MPMV 65S RNA also released three low molecular weight RNA species.

Radioactive DNA (^{3}H-cDNA) complementary to the RNA of Mason-Pfizer Monkey Virus was used in molecular hybridization experiments to demonstrate sequence homology between MPMV RNA and RNA of human malignant breast tumors. Hybridization products were analyzed by cesium sulfate equilibrium density centrifugation and hydroxylapatite chromatography. Twenty-seven of thirty-nine human malignant breast tumors tested contained MPMV-related RNA while no sequence homology was found in RNA from human benign breast tumors or normal breast, spleen, liver or kidney. Hybridizations between MPMV ^{3}H-cDNA and human breast adenocarcinoma RNA showed a $C_rt1/2$ of approximately 2 x 10^4. Thermal analysis of the hybrids demonstrated a T_m of approximately 80°C. Hybridization of radioactively labeled MPMV 60-70S RNA of breast tumor DNA is in progress.

Particulates with the properties of cores and/or ribonucleoproteins of RNA tumor viruses have been isolated from Sterox-SL-treated fractions of murine and human mammary adenocarcinomas. These particulates have an RNA-directed DNA polymerase, a 60-70S RNA, and a density of 1.26 g/ml or greater in sucrose equilibrium density gradients. Their uniquely higher densities lead to banding in regions comparatively free of cellular contaminants. These circumstances minimize some of the technical complications of performing the simultaneous detection assay in the presence of cell debris. Further purification and characterization of the reverse transcriptase from human malignant breast tumors, human milk, and human nipple discharge is now in progress.

Significance to Biomedical Research and the Program of the Institute: These studies are aimed at the isolation and characterization of a putative breast cancer virus from human milk and malignant breast tumors.

Proposed Course: The activities described will be continued.

Date Contract Initiated: 20 July 1973

Current Annual Level: $593,764

MICHIGAN CANCER FOUNDATION (NO1-CP-33347)

Title: Studies on Milk in High Risk Breast Cancer Families

Contractor's Project Director: Dr. Marvin Rich

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: The aims of this research program include characterization of the 734B particle being synthesized by the human breast tumor cell line MCF-7 and the identification and characterization of high-risk breast cancer populations, seeking to provide approaches to early detection and control of breast cancer. Animal and human model systems have been utilized to elucidate the role of viruses in the carcinogenesis of human breast cancer. The following results represent the major findings during this period.

Major Findings:

A. HUMAN MILK STUDIES

A1. RNase Destruction of Viruses in Milk. Previous reports have shown that RNase in human milk interferes with the detection and quantitation of reverse transcriptase containing particles of viruses. It was determined that mouse milk also contained RNase which apparently did not interfere with MuMTV or MuLV in those milks. This study showed that RT detection in mouse or human milk (both of which contain RNase) depends primarily on the amount of virus present. If there is enough virus to exceed saturation of the RNase present, there will be RT detection proportional to the amount of virus in excess. Since the number of putative human milk virus is very low in most milks, only limited studies may be carried out until alternative methods are deviced.

A2. Two Types of RNase in Human Milk. Preliminary experiments using isoelectric focusing show that there is in human milk one RNase with an alkaline isoelectric point and another with a neutral value.

B. HUMAN VIRUS STUDIES

B1. Possible Relationship Between 734B Virus and MuMTV. Reactivity of MCF-7 Cells with Anti-MuMTV Sera. Preparation of a hyperimmune, monospecific polyvalent antiserum to MuMTV has permitted close scrutiny of the relatedness of 734B and MuMTV. Comparison by immunofluorescence of 734B producer clones of MCF-7 cells with non-producer clones, human

epithelium cells, and BALB/c cells from MTV-free mammary glands or BALB/c cells infected with MuLV, showed no reactivity with the control cells and 5-7% reactive cells in the 734B producer cultures. The positive cells showed granular fluorescence in the cytoplasm. The fact that the hyperimmune serum reactivity could be absorbed out with solubilized 734B proteins further suggests that the cross-reactive antigen is a structural protein of the 734B particle.

B2. Reactivity of 734B Particles with Anti MuMTV Sera. Attempts were made to isolate MuMTV/734 B cross-reactive proteins by affinity column chromatography. While five polypeptides can be resolved in SDS-acrylamide gels from MuMTV preparations specifically bound to the anti-MuMTV affinity columns, only two have been resolved from the specifically-bound 734B proteins eluted from anti-MuMTV columns. This data, combined with the failure to identify the major MuMTV proteins (52,000 molecular weight) in the 734B preparations, suggests that 734B and MuMTV are not identical.

B3. Biological Characterization of 734B. To expedite the rapid and thorough characterization of 734B and its biological significance, 734B synthesizing cell cultures have been distributed to 7 collaborating laboratories as arranged by the Breast Cancer Virus Segment of the Virus Cancer Program.

B4. Nucleic Acid Hybridization Studies Show 734B Particles to be of Human Origin. Hybridization of ^{3}H-DNA copies of the viral genome 734B to human DNA and to DNA of tissues from animals that might have been sources of viral contamination was studied. Purified radioactive DNA probe was purified and then hybridized with DNA from human placenta, mouse liver, bovine thymus, cat liver, monkey liver, and hamster liver. Bacterial DNA was also hybridized with viral DNA under identical conditions to measure non-specific binding. Using the most stringent criteria, the DNA of the 734B virus particle showed 28-35% double-strandedness complementarity with human placental nuclear DNA. Average values of 5% were obtained with the control tissues. These results were very similar to those obtained with a control system using mouse liver and MuLV.

Significance to Biomedical Research and the Program of the Institute: These studies concern the isolation and characterization of a potential human breast cancer virus.

Proposed Course: The activities described will be continued at the current level.

Date Contract Initiated: 20 June 1971

Current Annual Level: $766,265

NETHERLANDS CANCER INSTITUTE (NO1 CP 33368)

Title: Immunogenetic Studies on Breast Cancer and Leukemia

Contractor's Project Directors: Dr. L.M. Boot and Dr. J. Hilgers

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To study the immunogenetics of mammary tumors and MTV transmission, as well as that of leukemia and MuLV, to determine the presence and function of specific genes for transmission, MTV replication, histocompatibility, and immune response.

Major Findings: Transmission genes and genes influencing MTV replication. Linkage experiments are well underway for GR-MTV (Exogenous) with testing completed for over twenty markers. The genetical analysis to determine the number of genes which control induction of C3Hf-MTV (NIV Endogenous) and whether NIV induction is linked to GR-MTV induction has begun. Important findings were that the extremely high expression of MTV-gs antigen in the genital tract organs of GR mice might offer an explanation for the fact that BALB/c females, if caged with GR males, become positive for MTV-gs antigen in their milk. Another finding was that young adult male mice are suitable for studying the induction gene of MTV since they can be selected according to whether they are expressing MTV-gs antigens in extracts of the coagulating gland and the epididymis.

Histocompatibility and/or Immune Response Genes. Investigations with congenic lines on the C57BL/10 ScSn background have shown that the incidence of mammary tumors after administration of C3H-MTV is dependent on the histocompatibility locus H-2. Their tumor incidence showed remarkable differences. The haplotypes H-2^b and H-2^m were highly resistant, those of H-2^t, H-2^d, H-2^l and H-2^a were highly susceptible with other haplotypes showing intermediate susceptibility. Thus, through recombinant experiments, it appears that the D-end of the H-2 locus determines susceptibility, the subloci $2D^b$ and $2D^g$ resistance, and $2D^d$ and $2D^f$, high susceptibility.

MTV-induced Cell Surface Antigens. Studies concerning the characterization of the MTV-induced cell surface antigens were begun to aid in the understanding of gene transmission and gene action. Currently a transplanted GR leukemia of thymic origin which shares the MLr, Thy. 1.2, and H-2.8 antigens with MTs is being used for these studies. The MLr antigen is also present in purified B-particle envelope and a glycoprotein of 52,000 daltons. The methodology for accurate and rapid detection of these antigens on various test cells was worked out.

Virological studies showed that the oncornavirus reported by Gelerblom et al did not cross-react with MTV or MuLV but cross-reacted with Mason Pfizer MV by virtue of two common antigens (16,000 and 28,000 Daltons respectively) found in both viruses. Additionally, virus-like particles with type C characteristics were detected by EM in 14 out of 16 cell cultures obtained.

from different cattle with hyperlymphocytosis. Nine detailed reports of these findings were submitted for publication during this contract period.

Significance to Biomedical Research and the Program of the Institute: This type of genetic study concerning the host's susceptibility and capacity for internal control of oncogenic virus expression and infectivity is of crucial importance to the program of this institute. The results obtained thus far begin to delineate a variety of factors in addition to the presence of an oncogenic virus, which contribute to or modify the pathogenesis of mammary cancer of leukemia. The findings described contribute to the investigations of possible etiological agents of human cancer, as well as to greater understanding of the fundamental biology of cancer.

Proposed Course: The contractor proposes to continue his studies concerning the genetics of MTV and MuLV expression, transmittability, susceptibility and their relationship to histocompatibility and immune response.

Date Contract Initiated: 28 June 1972

Current Annual Level: $50,200

NAVAL BIOMEDICAL RESEARCH LABORATORIES (NO1-CP-53502)

Title: Human Mammary Tumor Virology

Contractor's Project Director: Dr. Adeline Hackett

Project Officer (NCI): Dr. Robert Bassin

Objectives: This project is devoted to the development of human mammary epithelial cell substrates representing various clinical phases from normal to malignant and to the use of these substrates to study the etiology and control of human breast cancer.

Major Findings: A collection program for human breast fluids obtained by the Sartorius aspirator has been established at Peralta Hospital, Oakland. To date, more than 1,000 donors, all except 4 have yielded normal cells (determined by the Papanicolaou stain). A collection program for both benign and malignant breast tumors has been established at Alta Bates Hospital, Berkeley. Eight malignant and fourteen benign tumors have been studied.

Since these specimens are collected with the ultimate aim of developing breast cell lines for use in viral studies, they are routinely screened for virus production in primary culture by the reverse transcriptase assay. If there is indication of a positive enzyme assay, specimens are then examined by radioisotope density gradient centrifugation and electron microscopy.

All specimens are further characterized by growth rates in various media and by morphology with light, transmission, and scanning electron microscopy. Papanicolaou stains of both breast fluid and spilled tumor cells are used as the basis of clinical classification of each specimen.

Progress to date of this recently-established contract is primarily in the organization of specimen collection and development of techniques for specimen analysis. In vitro studies of these cells are in progress.

Significance to Biomedical Research and the Program of the Institute: The establishment of human breast cell lines will material with which vital questions relating to the viral etiology of human breast cancer can be answered. These questions cannot be resolved definitively using in vivo derived material. This activity is virtually essential to several other studies aimed at the detection and isolation of viruses or viral products in human breast tumor cells. These efforts fit the aims of the NCI.

Proposed Course: To continue the activities described.

Date Contract Initiated: 01 September 1974

Current Annual Level: $75,046

PFIZER, INC. (NO1-CP-33239)

Title: Viral Studies of Animal and Human Breast Cancer

Contractor's Project Director: Dr. Sami A. Mayyasi

Project Officer (NCI): Dr. Robert Bassin

Objectives: The principal aim of this contract is to study the role, if any, of MPMV as a tumorigenic virus and its relationship to simian and human breast cancer. The following are the most interesting results obtained during this reporting period.

Major Findings: KC cells form massive syncytia when co-cultivated with M-PMV infected cells. This phenomenon can be used as the basis of a rapid end-point assay for M-PMV and related viruses such as the AO and J-96 viruses recently isolated from human cell lines. Efforts are continuing to refine this assay system further so that enumeration type assays, comparable to the XC test for mouse leukemia virus, can be developed.

A radioimmunoassay for the purified major core protein, p27, of MPMV, has been developed and has been used to demonstrate the antigenic relatedness to MPMV of both monkey and human viral isolates. Additional radioimmunoassays for other MPMV associated polypeptides are being developed. In addition, a new low molecular weight, MPMV-associated polypeptide has been detected and is currently being characterized. Human tumors are being screened for antigens related to MPMV p27 by the radioimmunoassay described above. Also, in a preliminary study, human serum samples have not contained detectable levels of antibody to MPMV p27.

MPMV induces soft agar colony formation by monkey foreskin (FS-2) cells. The properties of cells isolated from the colonies, in particular their ability to induce tumors in vivo, are being characterized. In the initial

study tumors were induced in nude mice following inoculation of these cells. The tumors were grown in vitro, and the resulting cell line was shown to contain monkey cells.

Cloned stocks of MPMV, some of which have already been isolated, are being studied in order to examine the biological interactions, if any, that occur between MPMV and SSV-1-related C type viruses. Preliminary data indicate that MPMV may require interactions with other viruses for infectivity.

Significance to Biomedical Research and the Program of the Institute: A determination of the role of MPMV in breast carcinoma of monkeys is of obvious significance in assessing the role of virus in the human disease.

Proposed Course: This contract will be terminated on 9/30/75.

Date Contract Initiated: 28 June 1967

Current Annual Level: $200,000

RADIOBIOLOGICAL INSTITUTE (NO1-CP-43328)

Title: The Study of the Release of RNA Tumor Viruses and Its Genetic Control

Contractor's Project Director: Dr. Peter Bentvelzen

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: The principal aim of this program is to carry out studies in vivo as well as in vitro to elucidate the role of hereditary factors in the expression of genetically transmitted mammary tumor viruses (MuMTV) of mice. The importance of such genes in spontaneous mammary carcinogenesis will be evaluated in relationship to environmental agents such as chemical carcinogens and ionizing radiation. Some studies will also include the role of hormones in the control of these endogenous viruses.

Major Findings: The emphasis during the first year has been placed in the development of production of individual proteins and monospecific antisera. Another step has been the refinement and adaptation of the Sepharose-bead immunofluorescence assay for the detection of antigens and antiviral antibodies and refinement of an immunofluorescence double staining technique that allows simultaneous detection and discrimination of type B and type C viral antigens.

A quite interesting result has been the induction in vitro of MuMTV by dexamethasone from cells of the low mammary cancer mouse strains C57BL and BALB/c. MuMTV is induced in vivo in these strains with difficulty. These data suggest that possibly all strains of mice carry MuMTV and that humoral and other factors control the release of endogenous virus in these studies.

Significance to Biomedical Research and the Program of the Institute: The expression of MuMTV in non-mammary gland tissue and the effect of co-carcinogens of the expression of MuMTV is extremely important if we are to understand the pathogenesis of a putative human breast cancer virus. Furthermore, the determination of the presence or absence of a provirus for breast cancer is important to our understanding of the methodology projected for the prevention or control of this disease.

Proposed Course: To continue the activities described.

Date Contract Initiated: 26 April 1974

Current Annual Level: $122,500

TEL AVIV UNIVERSITY (NO1-CP-53517)

Title: Vaccination of Mice with Cell Free Antigens Derived from MMTV and A Particle

Contractor's Project Director: Dr. Asher Frensdorff

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To test whether it is possible to immunize mice against mouse mammary tumor virus (MMTV) without exposing them, in the process, to structurally intact virus. The contractor will investigate whether immunization of mice with individual, purified structural proteins from MMTV can decrease or abolish their susceptibility to spontaneous as well as virus-induced mammary tumors. Isolation and purification of the structural proteins will be performed under the mildest possible conditions thus ensuring conservation of the native conformation of the proteins. Immunization procedures are designed in such a way as to (a) increase as much as possible the immunogenicity of the proteins of which only very small doses are to be injected; (b) stimulate a cell mediated, rather than humoral, immune response.

Major Findings: This contract was only recently established. MTV-free (BALB/c) as well as vertically infected C3Hf and RIIIf mice are to be used. The effect of immunization with individual antigens is to be assessed on the incidence, both of spontaneous and of infectious virus-induced-tumors. The incidence of spontaneous tumors in unimmunized mice of the selected strains is such as to make detection of decreases in tumor incidence possible. With respect to the mouse strains used, the design of the experimental groups is such as to make maximal use of the cross reactivity between the viral strain from which the antigens are isolated (MTV-P from RIII milk) and the endogenous virus of the experimental animals. Thus, the possibility that cross-reactivity at the antigenic level may confer cross-immunity will be tested.

Significance to Biomedical Research and the Program of the Institute: The demonstration of immune protection with purified viral proteins is an important goal of the NCI program in tumor immunology. These studies will identify those virus fractions which have protective capacities and the methodology developed with this oncogenic virus will likely pave the way to similar work with a possible human cancer virus. Work with this virus is especially important since it provides a model system for solid tumor immunity which may be somewhat different from the existing leukemia models. Because of the demonstration of infectivity by viral nucleic acids, an immunizing fraction free of nucleic acid appears to be greatly desirable.

Proposed Course: The contractor hopes to demonstrate that a vaccination procedure that uses purified viral antigens and thus no structurally intact virion can protect MTV free mice. Moreover, the contractor hopes to show that even neonatally MTV infected mice can be protected by immunization with antigens derived from a viral cross reacting with their own.

Date Contract Initiated: 10 October 1974

Current Annual Level: $59,952

TEL AVIV UNIVERSITY (NO1-CP-23237)

Title: Isolation, Purification and Propagation of B type Particles from Human Milk in Israel

Contractor's Project Director: Dr. Iafa Keydar

Project Officer (NCI): Dr. Wade Parks

Objectives: To isolate and grow possible type B virions from the milk of breast cancer patients or from human adenocarcinomas using human embryonic tissue culture cells as substrates; to develop methods for the assay and enhancement of production of these "biochemical viruses"; and to continue characterization studies of the biochemical virions produced in human cell cultures and their relationship to mitochondria.

Major Findings:

1. The AV1 and AV3 cell lines that we used for infection of the human milks was shown to be HeLa cells by Dr. Walter Nelson-Rees. This could account for the detection of biochemical viruses due to Mason-Pfizer viruses known to be indigenous to certain strains of the HeLa cell line. Whether this is in fact the explanation is a subject for further investigation. Other cell lines developed by Dr. Keydar from embryonic mammary gland buds have been subjected to radiation and analyzed for virus production. One of these, 37-2, following induction by DMSO 0.5 to 2% and co-cultivation gives a positive simultaneous detection assay which measures biochemical viruses, Dr. Keydar is employing electron microscopy for the measurement of viruses. So far none have been detected.

2. The electron microscopic studies have revealed the presence of numerous dense bodies within mitochondria. The significance of these intramitochondrial bodies is not clear at the present time but is of potential significance if such structures could be related to viruses.

3. Using *[^{3}H]-uridine, the conversation of counts banding at 1.16 to structures banding at a density of between 1.22 and 1.26 in sucrose density gradients, was studied to detect further evidence of the viral nature of the human particles.

Significance to Biomedical Research and the Program of the Institute: The evidence of virus-like materials in human milk obtained from breast cancer patients is of obvious significance. However, the findings have not been developed or confirmed in this or other laboratories in the past 18 months in spite of demonstrated ability to work with the murine type B viruses and improved methodology for the identification and measurement of all RNA-containing reverse transcriptase positive viruses.

Proposed Course: Extend the contract for 3 months and characterize cell lines used by this contract in an effort to resolve the identity of the putative viruses in these cell lines.

Date Contract Initiated: 23 March 1972

Current Annual Level: $88,100.00

2. DEVELOPMENTAL RESEARCH SEGMENT
July 1, 1974 - June 30, 1975

The mission of the Developmental Research Segment is the elucidation of the association and presumed etiological relationship of RNA and DNA viruses to human neoplasia leading to the development of measures for the prevention or control of virally-induced neoplasms in humans. To meet this objective, contract research projects administered by this segment include studies on: seroepidemiology; virus gene expression in host cells; factors that influence virus gene expression; molecular processes in virus replication and cell transformation; and host responses to virally-induced antigens. During the past year, twenty-six projects contributed research data to the Virus Cancer Program through this segment.

Among the viruses suspected to be involved in the etiology of some human neoplasms, the most intensively studied is the Epstein-Barr virus (EBV). This virus infects the marrow-derived B-lymphocytes. The infected cells acquire the capability for unlimited growth in vitro. The thymus-derived T-lymphocytes are resistant to infection. In studies conducted at the Karolinska Institute and collaborating laboratories during the past year, it became evident that the perpetuation of B-lymphocytes in culture was not necessarily dependent upon chronic infection by EBV. In fact, one EBV-free line was originated from a Burkitt's lymphoma diagnosed in an American, who was sero-positive for antibodies against EBV. The malignant lymphoma cells from this patient were transplanted into "nude" mice. The B-cell malignant growth in this animal, explanted into culture, was found to be free of EBV DNA and the virus-induced nuclear antigen, EBNA.

Elevated humoral antibody titers against EBV antigens observed in patients with different neoplastic diseases have suggested the possibility of a causal relationship. It has now been shown that the high anti-EBV antibody levels in Hodgkin's disease patients in all probability are secondary to a T-lymphocyte depression in these patients. The highest elevation of antibody titer against EBV-induced antigen is associated with the sarcomatous, or lymphocyte-depleted, form of the disease which has a relatively strong impairment of delayed hypersensitivity, a T-cell immunological mechanism. This suggests that under normal conditions, T-cell function restricts the proliferation of EBV-carrying cells and thereby the antigenic stimulus inducing increased humoral response. A pertinent study relevant to this point was reported from Italy wherein two children with congenital thymic immunodeficiency syndromes were found to have high anti-EBV serum titers. Thymic grafts in these subjects resulted in the establishment of a normal T-cell picture and rapid normalization of serum titers to EBV capsid antigens (VCA) and early antigens (EA).

A causal relationship between primary EBV infection and the development of infectious mononucleosis in teen-aged individuals is now accepted. At the University of North Carolina, as well as in other laboratories, nucleic acid hybridization methods applied to the circulating lymphoid cells in the acute

phase of this disease failed to detect the presence of intracellular EBV DNA, possibly because T-cells predominate, and the sensitivity of the test procedure may have been insufficient to detect the levels of EBV DNA in the B-cell component of the specimens examined. Cell-mediated immunity studies revealed that the T-cells of persons with mononucleosis show a strong EBV-specific cytotoxicity detectable after the removal of the non-specifically cytotoxic complement receptor-positive cells. The locus of the antigenic stimulus engendering the immune response is unidentified.

Epidemiological studies had demonstrated an association between EBV-infection and nasopharyngeal carcinoma, and epithelial cell malignancy with heavy lymphocytic infiltration. Since only B-lymphocytes were known to be susceptible to infection by EBV, it appeared unlikely that the virus genome had entered the neoplastic cells. Using tumor sections, in situ nucleic acid hybridization methods applied by zur Hausen provided strong evidence that the EBV DNA was located in the malignant epithelial cells. Recently, the carcinoma cells, freed of lymphoid cells by passage through "nude" mice, were unequivocally shown to contain virus DNA and the EBNA antigen. Furthermore, the lymphoid cells infiltrating the tumor were found to be predominantly T-cells.

Among all lymphoproliferative malignancies, the regularity with which EBV DNA is detected in the neoplastic tissues of histologically confirmed African Burkitt's lymphoma indicates a unique relationship to that neoplasm. The presence of the virus-induced nuclear antigen, EBNA, parallels the presence of viral DNA in cells, and is a reliable indicator of infection. Strain differences between EBV isolates have been observed, and one strain, B95-8, had been shown by Miller to induce lymphoproliferative disease in marmosets. Although overt neoplasia has not been observed in other species of monkeys infected with EBV, in recent tests lymphocytes obtained from squirrel monkeys, infected with EBV in vitro and then re-introduced in quantity into the donor animal, grew and killed it, indicating their malignant state. It is probable that this primate host established an effective immunological control of the outgrowth of lymphoid cells after inoculation with virus in vivo but could not cope with the numbers of cells introduced after their transformation in vitro.

Seroepidemiological studies have provided evidence of an association between herpes simplex virus type 2 (HSV-2) and invasive carcinoma of the uterine cervix. Data acquired at Baylor College of Medicine from tests using sera from carefully matched cases and controls support the existence of a significant relationship between serological evidence of infection and the presence of the invasive cancer. Efforts are being made to improve the serological procedures for detecting past infection by this virus. Additionally, attention is being directed to the virus-induced antigens expressed in tumor-bearing individuals and in cultured HSV-2 transformed cells. From Johns Hopkins, a good correlation has been reported to exist between the presence of cervical cancer and the presence in the patient of antibody reacting with an antigen (AG-4) produced within the first few hours after HSV-2 infection of Hep-2 cells in vitro . The reactive antibody is said to be IgM and, therefore, different from co-existing antibodies to the other virus-induced antigens. VP134, a 134,000 molecular weight non-structural,

virus-induced protein isolated at the Baylor College of Medicine, has been detected in some HSV-transformed hamster cell lines. VP134 is found in both HSV-1 and HSV-2. Current information indicates that a higher proportion of the sera from cervical cancer patients reacts with this protein than is observed with sera from controls free of this neoplasm.

At the University of North Carolina the search for HSV-2 DNA in human cervical cancer tissue, using nucleic acid hybridization methods has been largely unrewarding. Since only a small portion of the virus DNA may be retained within the cancer cell, the procedures for detection may not have been sufficiently sensitive. Studies at the Pennsylvania State University demonstrating the transformation of cultured animal cells by UV-inactivated HSV-2 *in vitro* suggest that different isolates vary in their capability to induce these cell changes. Therefore, it is possible that the strain of HSV-2 used to prepare the complementary nucleotide sequences as probes may lack the sequences present in the cancer cell. At this time, the inability to detect the virus DNA in the cervical tumor tissue does not eliminate HSV-2 as a possible factor in the etiology of this neoplasm.

Cebus monkeys had been found to respond to HSV-2 infection of the genitalia much as do humans. Accordingly, this species was selected as an experimental model to determine whether repeated virus infection *in vivo* would result in the development of cervical neoplasia. To date, abnormal cytological diagnoses have been made on cervical smears from 2 control and 53 virus-inoculated animals. Six of these infected monkeys have had more than 4 sequential abnormal smears persisting from 10 to 28 months. In 4 of the animals there is an apparent progression of changes.

The interactions between an oncogenic herpesvirus and oncogenic RNA viruses have been under study in an avian system. A Marek's disease herpesvirus (MDHV) preparation free of avian leukosis virus (ALV) was used in ALV-free chickens at Life Sciences, Inc. When different subgroups of ALV were administered concurrently with MDHV, a higher mortality was observed in these birds than in birds infected with either virus alone. In the dual infections, the recovery of MDHV from skin and feather follicle epithelium was reduced, and the titer of ALV in organs was increased. Washed lymphocytes in the presence of autologous serum from dually-infected chickens showing minimal tumor development were markedly cytotoxic for MDHV-infected chicken embryo fibroblasts. In contrast, sera from chickens that developed the extensive nodular lymphomatous masses characteristic of contact exposure to MDHV infection alone blocked cytotoxicity of sensitized lymphocytes for target cells.

The virus particles morphologically resembling Type C RNA virus that were first detected in the placental tissue of baboons at the Southwest Foundation for Research and Education have been observed in the perivitteline space and zona pellucida of the follicular oocyte. A number of isolations were made from placentas and from embryonic tissues providing added evidence for the vertical transmission of these viruses. Similar placental particles have been detected in other primate species including humans. Their significance in oncogenic processes in primates is unknown.

The studies at Columbia University which demonstrated the presence of cytoplasmic particulates with a reverse transcriptase activity and with RNA nucleotide sequences showing homology to sequences in known RNA tumor viruses were expanded to show that nucleotide sequences unique to cancer tissues were not detected in their normal tissue counterparts. This was interpreted to signify that the cancer-related sequences were not vertically transmitted from parent to progeny. The original observation made on leukemia was shown to include lymphomas, carcinomas and tumors of the central nervous system.

Poly A-positive RNA extracted from human leukemia cells at the Hopital St. Louis in Paris was examined for homology with DNA probes synthesized from Moloney sarcoma/leukemia virus RNA. Under the experimental conditions used, the majority of the case materials examined showed no more hybridization to the C-DNA than did RNA extracted from normal leukocytes. Specimens from two cases, an acute lymphocytic and an acute myelogenous leukemia, showed a high degree of hybridization. These must be subjected to further evaluation. In those cases where the findings were negative, the possibility exists that some sequences were missed only because of the relatively low amounts of the RNA used in the hybridization tests.

Cytoplasmic particulates recovered from quantities of acute myeloblastic leukemia cells at Litton Bionetics, Inc., contained a reverse transcriptase activity, but did not resemble virions morphologically. Radioisotope-labelled DNA synthesized by an endogenous reaction of these particulates hybridized to a highly significant degree with RNA of both the woolly monkey sarcoma virus and the Kirsten murine sarcoma virus grown in normal rat kidney cells. The reverse transcriptase from these particulates was shown to exist in two forms interconvertible with respect to their molecular weights of 70,000 and 150,000. Since the two forms differ in their biochemical characteristics, interconvertibility may be associated with some regulatory function of polymerase activity.

The major proteins and glycoproteins of the Friend (FLV) and Rauscher (RLV) murine leukemia viruses have been isolated and characterized in two laboratories. The gp70, a glycoprotein component of about 70,000 molecular weight and the p30 and p15, proteins of 30,000 and 15,000 molecular weight respectively, each differ antigenically. However, each carries virus type, group and interspecies determinants.

At the Albert Einstein College of Medicine, radioimmunoassay procedures using antiserum to the RD114 cat virus and RLV p30 antigen detected a class of interspecies determinants common to murine, feline and nonhuman primate viruses. Antigen with the same apparent affinity for antibody binding as that of the interspecies determinants of the highly purified p30 RLV core protein was found in relatively high concentrations in the tissues from patients with systemic lupus erythematosis, in some neoplastic tissues, and also in normal human tissues. The competing protein extracted from the spleen of a lupus case, chromatographed on phosphocellulose, showed a size fractionation during gel filtration similar to known p27 - p30 viral proteins.

At the Duke University Medical Center the cytotoxic action of a monospecific antiserum for FLV gp71 exerted against human myelogenous leukemia cells suggested the presence of cross-reacting cell surface antigens. The cytotoxic reaction could be blocked by virus gp71 and by the positive target cells but not by normal or lymphatic leukemia cells. This work supports the observations reported by the Litton Bionetics group actively studying virus-related components recognized in myelogenous leukemia cells.

The high incidence of neoplasia in human organ graft recipients stimulated related studies in murine systems at the Massachusetts General Hospital. It was observed that Type C RNA virus was activated by the graft versus host reaction leading to the development of malignant lymphoma in as many as one-half of the animals. Treatment with interferon inhibited virus activation and prevented development of lymphomas. Immune suppression in animals receiving skin grafts from animals even with only minor histocompatability differences considerably increased virus activation in comparison to either treatment alone. Both mouse-tropic and xenotropic viruses were activated. The observations indicated that the detection of Type C virus activation following immunostimulation reactions might depend on persistent immuno-suppression following the induction event. That is, although lymphocyte responses against histoincompatability antigens are sufficient to induce Type C viruses, their continued replication and the eventual development of neoplasia may depend on an ineffective mechanism for virus elimination. The studies showed that (BALB/c x A/J)F_1 mice can develop cell-mediated immune responses against antigens on syngeneic cells infected with endogenous mouse-tropic Type C viruses. It appears that at least some strains of mice may be exposed to antigens of Type C viruses at an early age, prior to detectability of infectious virus in these animals, and that they can respond with both humoral and cell-mediated immune responses against these antigens. Variations in the production of these immune responses may markedly influence the liklihood of Type C virus replication and subsequent oncogenesis.

The stimulation of Type C virus production by dexamethasone and inhibition by interferon studied at Litton Bionetics, Inc. was shown to be a post-transcriptional effect on the gene expression of RNA tumor viruses. It was found that this treatment did not alter the amount of intracellular viral protein, suggesting that dexamethasone and interferon act at the step after the viral proteins are synthesized, perhaps at the assembly of virus particles.

Studies on purified, characterized viral proteins conducted at the Albert Einstein College of Medicine and at Duke University showed that the major virion component involved in the neutralization of RLV and FLV infectivity is the 69,000 - 71,000 virion envelope glycoprotein. Although antisera produced against 15,000 and 30,000 molecular weight Friend virus proteins did not effectively neutralize virus infectivity, they did induce target tumor cell lysis in the presence of complement. This suggests that they also are expressed on the infected cell membranes.

Earlier studies at the Ohio State University Research Foundation demonstrated that tumor homogenates containing inactivated feline sarcoma virus provided

protection against sarcoma virus challenge. Cats with serum neutralizing antibody to the sarcoma virus passively transmit immunity to sarcoma virus challenge in their offspring. Attempts to actively immunize kittens with a sarcoma virus vaccine yielded a variable virus-neutralizing humoral antibody response without activity against feline virus tumor cell-associated cell membrane antigen. Current interest is directed to the virus-infected cell membrane antigens for effective protection against virus challenge.

Dr. Robert A. Manaker, VBB, Division of Cancer Cause and Prevention, Chairman
Dr. Maurice L. Guss, VBB, Division of Cancer Cause and Prevention, Vice-Chairman

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP3-3311)

Title: Research Studies of the Molecular Biology of Oncogenic Viruses and Malignant Transformation

Contractor's Project Director: Dr. J. Thomas August

Project Officers (NCI): Dr. George F. Vande Woude
Mr. J. T. Lewin

Objectives: To determine the molecular events involved in the adsorption and penetration of RNA tumor viruses into host cells, in viral nucleic acid replication, and in malignant transformation of cells.

Major Findings: The major envelope glycopeptides gp69/71, major core protein p30, and the p15 viral protein were purified from Type C viruses. Studies showed that (i) each expressed multiple antigenic determinants: type, group and interspecies; (ii) that the gp69/71 is the major envelope constituent, corresponds to the cell surface G_{IX} antigen, and is a major component active in viral neutralization; (iii) that the gp69/71 was expressed by a high percentage of NRK cells nonproductively transformed by Kirsten sarcoma virus; and (iv) that the p15 corresponds to the FMR cell surface antigen. The gp69/71 and p30 could be detected in tissues of all mice examined except strain 129.G_{IX}^- and NZB mice showed an extremely high serum concentration of gp69/71.

A component with physical, chemical and immunologic properties of a primate virus p30 protein was found in both normal and malignant tissues of human subjects and in high concentration in tissues of patients with lupus erythematosus.

The RNase H activity co-purifying with avian myeloblastosis virus (AMV) reverse transcriptase was shown to be a progressive exonuclease acting from either 5' or 3' ends.

The analysis of intermediate steps in replication of viral RNA have shown the presence of covalently linked DNA·RNA hybrids of about 10^5 daltons.

Analyses of the mechanism of transcription of viral DNA to yield RNA are in progress utilizing isolated nuclei from infected cells and highly purified preparations of RNA polymerase. One percent of the RNA formed in vitro is found to be viral RNA and specifically requires the presence of chromatin isolated from the infected cells.

Significance to Biomedical Research and the Program of the Institute: The knowledge gained of molecular mechanism and products in tumor virus-cell relationships, the factors involved in the repression and derepression of virus gene expression, and the intracellular events associated with malignant transformation may be used to develop sensitive methods for detection of virus or virus activity in human cells and for devising a basis for therapy by blocking translational events at the subcellular level during cell transformation.

Proposed Course: Major attention will be given to the purification and characterization of virus coded envelope components and, in particular, those that are associated with the autogenous immune response of the host. Studies will be extended to analyze the possible relatedness of viral genetic expression to human diseases. Efforts will be directed toward the further characterization, isolation and mode of action of protein factors which permit AMV reverse transcriptase to transcribe or replicate long DNA chains.

A detailed attack on the mechanism of transcription of AMV sequences in chromatin will be undertaken. The influence of histone and polyanionic fractions derived from chromatin on this specific transcriptional process with eukaryotic RNA polymerase will be examined.

Date Contract Initiated: April 26, 1971

Current Annual Level: $396,120

BAYLOR COLLEGE OF MEDICINE (NO1-CP3-3257)

Title: Studies on Viruses as Related to Cancer

Contractor's Project Director: Dr. Joseph L. Melnick

Project Officers (NCI): Dr. Berge Hampar
Dr. Michael A. Chirigos

Objectives: To determine the relationship of viruses to selected neoplasias and their significance in the neoplastic process.

Major Findings: In studies of the relationship of herpesvirus (HSV) to cancer, an indirect radioimmunoassay followed by SDS-polyacrylamide gel electrophoresis of washed immune precipitates revealed that a significantly higher proportion of sera from cervical cancer patients (>90%) reacted with an early nonstructural polypeptide of HSV (VP134) as compared to sera from matched breast cancer patients (30%) or normal individuals (40%); the higher reactivity of the cervical cancer sera was independent of neutralizing antibody levels to HSV-2. Eleven cell lines established from cervical cancer biopsies are being examined for HSV-functional information.

The phenotype was studied of 6 oncogenic and 2 potentially oncogenic HSV-transformed hamster cell lines. Antiserum to HSV polypeptide VP134 was found to detect specific immunofluorescent antigens in some of the transformed

lines. Functional HSV genes were detected in HSV-2-transformed hamster cells through the enhanced replication of temperature-sensitive (ts) mutants of HSV-2 in such cells (but not in normal cells) at the nonpermissive temperature.

The preliminary phenotypic characterization was accomplished for 22 ts mutants of HSV-1 and 8 ts mutants of HSV-2. Intertypic complementation and recombination tests have led to the detection of gene functions shared by HSV-1 and HSV-2.

Antisera to several purified HSV-induced polypeptides have been obtained, some of which are capable of discriminating between HSV-1 and HSV-2 while others are capable of detecting specific antigens in HSV-transformed cells.

Herpesvirus saimiri that has been propagated at 38°C was found to be attenuated for marmosets.

Monkeys inoculated 2 years ago with hepatitis B (HB) antigen and the hepatic carcinogen DENA (diethylnitrosamine) began to develop hepatomas which are now being transplanted into "nude" mice and studied for the presence of HB antigen.

A block in the synthesis of the most complex fucolipids was found to be characteristic for cells oncogenically transformed by murine sarcoma virus, by papovavirus SV40, and by herpesvirus type 2. The same alteration in fucolipid synthesis was revealed by cultured human tumor cell lines tumorigenic for anti-thymocyte serum-treated mice and not by the nontumorigenic ones.

Significance to Biomedical Research and the Program of the Institute: Serological and sero-epidemiological evidence obtained by controlled studies on patients with squamous cell carcinomas implicate genital and oral strains of herpesvirus as factors in the genesis of these malignancies. Intensive study of the biochemical, genetic, and immunological aspects of the herpesviruses associated with these neoplasms provides fundamental information which may be invaluable as the role of the herpesviruses in oncogenesis is further developed.

Proposed Course: In order to solve the problem of the causal relationships of herpesvirus 1 and 2 to human carcinomas, the following objectives are being emphasized by the contractor: (a) detection of herpesvirus gene functions in human carcinomas by complementation and marker rescue studies; (b) search for virus-specific antibodies and antigens in human tissues with purified specific virion and nonvirion antigens of herpesviruses; (c) insights into cell transformation and genetics by temperature-sensitive and UV mutants of herpesviruses; and, (d) mechanism and definition of virus-induced alterations of cell membranes and function.

Date Contract Initiated: June 27, 1963

Current Annual Level: $548,000

BOSTON HOSPITAL FOR WOMEN (NO1-CP4-3379)

Title: Retrospective Cohort Study of Cervical Neoplasia and Herpes Simplex Virus Type 2

Contractor's Project Director: Dr. Lorna D. Johnson

Project Officers (NCI): Dr. Gary Pearson
Mr. J. T. Lewin

Objective: To provide serum specimens and historical, cytologic, and histopathologic data on the donors for investigations on the relationship between herpes simplex virus type 2 infections and cervical dysplasia or neoplasia in a cohort under study since 1959.

Major Findings: This project has been underway for an insufficient period for a significant report.

Significance to Biomedical Research and the Program of the Institute: The unique population from which the specimens are being drawn permits a "prospective" study to achieve more information concerning the relationship between HSV-2 and cervical cancer.

Proposed Course: This project to acquire specimens of serum from a defined cohort is a 2-year effort.

Date Contract Initiated: June 4, 1974

Total Level: $129,810

COLUMBIA UNIVERSITY (NO1-CP3-3258)

Title: Replication of Oncogenic Viruses and Its Relation to Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officers (NCI): Dr. Takis S. Papas
Mr. J. T. Lewin

Objectives: This project is directed toward the elucidation of the molecular biology of RNA tumor viruses and the mechanisms by which they induce oncogenic transformation of infected cells. This knowledge is applied to the determination of the viral etiology of human neoplasms and ultimately to development of effective control measures for human cancers.

Major Findings: The possibility that an interaction between an RNA tumor virus and Epstein-Barr virus may be the determinant of disease in African Burkitt's lymphoma is suggested by the detection of particles characteristic of RNA tumor viruses in the tumor.

H^3-DNA probes, endogenously synthesized by particles from Hodgkin's disease and Burkitt's and other lymphomas, were used to show that the nuclear DNA contained particle-related sequences that could not be detected in the DNA of normal cells.

Although normal and Rauscher murine leukemia virus-infected mouse (BALB/c) cells contain an equal copy number of murine viral sequences, spleen DNA from Rauscher murine leukemia virus-infected mice possessed a greater extent of homology with single-stranded Rauscher murine leukemia virus DNA product than did DNA from normal BALB/c mice. In addition, unique DNA sequences were demonstrated in spleen DNA of Rauscher murine leukemia virus-infected mice but were not detected in the DNA of normal mice.

The application of the simultaneous detection assay to carcinomas of the lung, colon, rectum, and stomach revealed postive evidence of 70S RNA in association with reverse transcriptase in 13 of 17 colon tumors, 3 of 5 stomach tumors, 3 of 3 rectal tumors, and 7 of 10 lung tumors. In contrast none of 25 corresponding normal tissues showed detectable evidence of particles containing 70S RNA and reverse transcriptase. In 5 of 8 specimens of human melanoma, 70S RNA was found in association with reverse transcriptase. The tritiated DNA probe from human melanoma did not hybridize with normal human skin RNA, nor with RNA from human basal cell carcinomas or squamous cell carcinoma.

In studies of the homology between oncornaviral genomes and cellular DNA of the natural host and related species, three general patterns of homology between viral DNA probes and nonrepeated cell DNA were observed: (1) Strong homology to normal DNA of the species from which the virus was isolated was noted for AMV, MMTV, RLV, FeLV, and RD114. (2) Weak homology to host normal DNA, but greater than to any other species, was noted for avian REV and MPMV. (3) Greater homology to a species other than that from which the virus was isolated was noted for SSV-1 (greater affinity for rodent DNA) and MSV-Harvey (greater affinity for rat DNA).

Strong evidence was obtained that MPMV may be related to a putative human breast cancer virus. No detectable homology of MPMV-DNA was observed with RNA from 11 human benign breast tumors, or normal human spleens, livers, or kidneys, but up to 25% hybridization with RNA from human breast cancer tissue with the hybrids having a Tm of 80.5C.

A method has been developed for purification of RNA tumor viruses from malignant tissue by a combination of isopycnic and isokinetic gradient centrifugation. This procedure permits improved separation of membranes from virus particles and has been used successfully with leukemic tissues and murine mammary tumors.

Significance to Biomedical Research and the Program of the Institute: A systematic molecular biological study is being pursued to determine the role of viruses in the genesis of human cancer. Studies demonstrated the presence in human cancers of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. New approaches to the study of virus-cell relationships have been devised. The contractor has developed data which are highly prevocative and his pioneering advances provide a

foundation for future investigation leading to an understanding of the virus-cell relationship as it applies to human cancer.

Proposed Course: Malignant human cells will be probed for the presence of nucleic acid sequences not present in non-neoplastic human tissues. Virus-like components of particulates extracted from cells of different human tumors will be compared and their relationship to similar components of RNA tumor viruses will be determined. Human mesenchymal tumor cells will be examined for the presence of antigens related to antigenic determinants of structural proteins of known Type C viruses.

Date Contract Initiated: October 29, 1969

Current Annual Level: $1,210,000

CORNELL UNIVERSITY (NO1-CP3-3346)

Title: Application of Feline Virus Systems in the Study of Viral Relationships to Human Neoplasia

Contractor's Project Director: Dr. Charles Rickard

Project Officers (NCI): Dr. Michael Chirigos
Mr. J. T. Lewin

Objectives: (1) To determine whether endogenous or exogenous Type C feline viruses are associated with leukemia or other neoplasms in children. (2) To determine whether treatment with a chemical carcinogen will induce Type C virus expression in cats or cat cells.

Major Findings: A search for virus of Type B morphology in feline mammary tumor was unsuccessful. No data were acquired associating the known feline viruses with childhood leukemia.

Significance to Biomedical Research and the Program of the Institute: The close contacts between children and household pets necessitate investigation of possibilities for infection by viruses recognized in these animals and for resulting disease.

Proposed Course: This contract was terminated on December 31, 1974.

Date Contract Initiated: June 23, 1965

Annual Level: $150,000

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP3-3308)

Title: Expression of the RNA Tumor Virus Genome in Animal and Human Malignant Cells

Contractor's Project Director: Dr. Dani P. Bolognesi

Project Officers (NCI): Dr. Peter Fischinger
Mr. J. T. Lewin

Objectives: To characterize the structural components of animal RNA tumor virus particles and to develop these as serological probes to detect virus gene products in animal and human malignancies and as agents for possible immunological control of viral infection.

Major Findings: Serological analyses of polypeptide p15 from Friend leukemia virus (FLV) revealed antigenic cross-reactivity with other mammalian Type C oncornaviruses. Antiserum to p15 of FLV reacted with intact murine leukemia viruses (MuLVs) in radioimmunoprecipitation (RIP) assays. The antigen-antibody complexes formed by this reaction were isolated and shown to contain p15 following analysis by SDS polyacrylamide gel electrophoresis. Natural antibody in mice to MuLV reacted in a similar fashion with p15, but in addition, also with the virus glycoproteins (gp71, gp45). Biological studies indicated that gp71, rather than p15, is principally involved in the virus neutralization reaction. These and other studies indicate that p15 is localized on the virus surface and is recognized by natural antibodies in mice to MuLV.

Cultured cells of different chemically-induced C57BL/6 murine sarcomas produced variable amounts of infectious MuLV and contained proportional amounts of MuLV structural components as determined by radioimmunoassay. Monospecific antisera directed against the major MuLV glycoprotein (gp71), the major internal antigen (p30), and the ribonucleoprotein (p10) were all capable of mediating tumor cell lysis in the presence of active complement, suggesting these viral structural components were localized, at least in part, at the cell surface. Membrane immunofluorescence studies with MuLV p30 antiserum confirmed surface localization. Addition of small quantities of MuLV p30 polypeptide to normal cells and tumor cells enchanced the cytotoxicity of MuLV p30 antiserum to those cells. This study establishes the presence of virion structural components on the cell surface (primarily gp71, p30), indicates that antibodies directed against these mediate cellular lysis in the presence of complement, and shows that the expression of such antigens can be independent of virus synthesis or cellular transformation. It could be shown further that the expression of p30 and gp71 could be non-coordinate.

Normal rat kidney (NRK) cells transformed by Kirsten murine sarcoma - leukemia virus (NRK-K) were synchronized by double thymidine block. At intervals after release from the blockage, the cells were examined for the presence and amount of the p10, p12, and p30 internal virion proteins using dilutions of antisera to p10, p12, and p30 for cytoplasmic immunofluorescence. Levels of p30 varied during the cell cycle with maximum expression late in S+ G_2 and minimum expression during M and G_1 phases. Expression of p30 was also coordinated to the cell cycle in NRK cells nonproductively infected with Harvey murine sarcoma virus (NRK-H). The presence of p30 on the surface of NRK-K cells was shown by immunoferritin electron microscopy and membrane immunofluorescence. Although virus production does not take place in NRK-H cells, p30 was demonstrable on the surface of these cells by membrane immunofluorescence. This study shows that the synthesis of the major internal

polypeptide of MuLV is synthesized during a very specific phase of the cell cycle, possibly in contrast to other virion polypeptides.

Leukemia-specific antigens have been isolated and partly purified from human cells in collaboration with R. Metzgar at Duke University. The material with leukemogenic activity cross-reacts partially with gp71 of MuLV and has similar physicochemical properties with gp71. This investigation will be intensively pursued in order to define the possible relationship between these molecules. Highly purified preparations of the leukemia-specific antigens will be obtained to establish a radioimmunoassay similar to those for gp71 and p30. Similar studies are in progress with recently isolated melanoma specific antigens and plans are being made to approach similar questions with tumors of the breast and of the brain. The major glycoprotein of MuMTV (gp52) has been isolated and the presence of cross-reactive material in breast cancer tissue will be determined.

Significance to Biomedical Research and the Program of the Institute: The virus genome is expressed by translation of message into protein products. This project was initiated to characterize the major protein antigens of selected RNA tumor viruses, prepare immune sera to identify these antigens in infected cells, and to utilize these materials to determine basic molecular events in the process of cellular transformation. The knowledge gained may be used to develop sensitive methods for detection of virus activity in human cells and to provide a basis for therapy by blocking translational events at the sub-cellular level to prevent cell transformation.

Proposed Course: Virion molecules in infected and uninfected cells will be characterized. Putative precursor polyproteins will be characterized and defects in protein synthesis in abortive virus infections will be determined. The immune response of the mouse to surface antigens of the virion (p15, gp45, gp71) will be studied to gain information concerning host defense against virus infection and pathogenesis. A systematic analysis of the leukemic process in the mouse and a detailed characterization of the material in human leukemic cells which cross-reacts with certain Type C virion antigens will be initiated. Studies will continue on the isolation and characterization of major structural components of the mouse mammary tumor virus.

Date Contract Initiated: March 1, 1973

Current Annual Level: $366,780

EMORY UNIVERSITY (NO1-NS2-2301)

Title: Oncogenic Potential of Herpesvirus in Monkeys

Contractor's Project Directors: Dr. Andre Nahmias
Dr. Zuher M. Naib

Project Officers (NCI): Mr. J. T. Lewin
(NINDS): Dr. John L. Sever

Objectives: To conduct cytologic and immunologic assessment of the response of Cebus monkeys to repeated genital infections by herpes simplex virus type 2 (HSV-2).

Major Findings: This project constitutes one part of a collaborative study with NINDS to determine the oncogenic potential of herpesvirus in an experimental primate animal. Animals on this project are held at Meloy Laboratories under contract NO1-NS2-2306.

During the year, about 3,700 virological specimens, 2,500 serological specimens and 2,000 cytological specimens were processed. From observations on the animals entered into this project, it appears unlikely that spontaneous cervical neoplasia occurs in the Cebus monkey. A mild cytological atypia was observed on one occasion in 2 controls and in 30 herpesvirus-inoculated animals. Of particular interest are 6 virus-inoculated animals with cytological abnormalities that have persisted for 10-28 months, 4 with apparent progression of the cytological changes. A comparison between colposcopic and cytological observations indicated a 77% correlation between these two techniques. An anti-complement immunofluorescence (ACIF) technique was adapted to detect HSV-2 antigens in human cervical cancer cells from biopsied specimens using hyperimmune rabbit anti-HSV serum, seronegative human serum as a source of complement, and a fluorescein-conjugated anti-human complement serum. HSV antigens were detected in two-thirds of 40 human cervical cancers tested with 2 to 54% of the cells showing positive fluorescence and in 10-25% of animal tumor cells transformed *in vitro* or *in vivo* with HSV.

Significance to Biomedical Research and the Program of the Institute: This project uses a nonhuman primate to provide data to help establish the nature of the apparent association of herpes simplex virus type 2 and human cervical carcinoma.

Proposed Course: This project is expected to continue for at least 3 more years.

Date Contract Initiated: March 1, 1972

Current Annual Level: $46,742

HOPITAL ST. LOUIS (NO1-CP3-3365)

Title: Molecular Virological Studies on Human Leukemia

Contractor's Project Director: Dr. M. Boiron

Project Officers (NCI): Dr. George Todaro
Mr. J. T. Lewin

Objectives: To search for unique viral nucleotide sequences in the DNA of human leukemic cells and for expression of the viral genome integrated in these cells using murine leukemia and sarcoma viruses grown in human and in mouse cells as a source of the synthetic DNA probe.

Major Findings: A systematic investigation of virus-like nucleotide sequences in human leukemic cells was conducted, using as the probe, single-stranded DNA synthesized by MSV-M (MLV) activated virions. The probe contained complete and almost uniform sequences of the entire 70S genome. Using direct hybridization of the single-stranded c-DNA with cellular poly A-rich RNA extracted from control or leukemic human cells, only one preparation from a case of acute lymphoblastic leukemia (ALL) was strongly positive with 36% hybridization. One case of chronic lymphocytic leukemia (CLL) and one case of chronic myelocytic leukemia (CML) were negative. Five cases of ALL, two of acute myelogenous leukemia and two of acute monoblastic leukemia were slightly positive with hybridization ranging from 2.4 to 5.5%. All normal controls were negative. When the technique of annealing c-DNA to sheared and denatured cellular DNA was used, the results were more promising in that higher levels of hybridization occurred with the three "leukemic" DNA's tested. Additional studies of c-DNA - RNA hybridization for 31 cases of acute leukemia showed only one highly positive result from a case of acute myelogenous leukemia. Simian sarcoma virus (SSV-1) was adapted for growth in a human embryonal rhabdomyosarcoma cell line in order to prepare a simian c-DNA probe.

Significance to Biomedical Research and the Program of the Institute: Data have been reported demonstrating the presence of nucleotide sequences in human leukemia cells homologous to sequences in RNA tumor virus RNA. An intensive study of human leukemia and Hodgkin's disease cells under this project is expected to provide information expanding on these observations to determine the nature of the common nucleotide sequences, their distribution in patients' cells, and the relationship between the presence of the common sequences in virions and the species and nature of the cells in which the virus is grown.

Proposed Course: The original and reproducible techniques developed will be applied to the investigation of as many human leukemias as possible.

Date Contract Initiated: June 28, 1972

Current Annual Level: $66,500

JOHNS HOPKINS UNIVERSITY (NO1-CP3-3345)

Title: Herpesvirus Antigens and Virions in Neoplastic Cells from Cervical Carcinoma

Contractor's Project Director: Dr. Laure Aurelian

Project Officers (NCI): Dr. Gary R. Pearson
Mr. J. T. Lewin

Objectives: The immediate objective of this project is the identification of herpes simplex virus type 2 (HSV-2) antigens and virions in neoplastic cells. The ultimate objective is development of evidence for or against HSV-2 as a factor in the etiology of carcinoma of the human uterine cervix.

Major Findings: The large scale study to determine the presence of antibody to early, tumor-specific HSV-2 antigen (AG-4) in sera from patients with cervical carcinoma and matched controls is in progress. It is expected that approximately 500 sera will be analyzed. Following therapy serum is drawn from patients in this study and from those in a previous study in order to determine the prognostic significance of AG-4 antibody. These studies cannot be analyzed and tabulated until all the sera received under code have been assayed. AG-4 antibody is being assayed in cases of primary herpes. Prior to AG-4 analysis, the sera are tested for neutralizing antibody to HSV-2 in order to ascertain the primary nature of the infection. To date, of the four sera assayed, none had AG-4 antibody. The virus host-cell relationship in male consorts of cervical cancer patients and controls is under study using coded specimens. These consist of serum, studied for antibody to HSV-2 (neutralization) and to AG-4 (complement fixation), and ejaculates, used to isolate herpesvirus by 6 blind passages on HEp-2 cells. To date, virus was isolated from 2 of 9 specimens. Two new cell cultures, still in early passage, were established from biopsies of invasive cervical cancer. Efforts at establishing new cultures from pre-invasive biopsies are now in progress. It appears that antibody to AG-4 is IgM and differs from antibody to other viral antigens. In infected HEp-2 cells, AG-4 is located on the cell surface. Studies are now in progress to identify AG-4 on the surface of cervical tumor cells.

A study of the possible role of estrogenic hormones as co-factors in oncogenesis indicated that their inhibitory effect on virus replication is due to loss of infectivity caused by the destruction of the viral envelope and perhaps mediated by the release of hydrolytic enzymes following hormone treatment.

Significance to Biomedical Research and the Program of the Institute: Considerable data has been acquired demonstrating an association between HSV-2 infection and carcinoma of the uterine cervix. In humans, it is extremely difficult to conclusively show that virus associated with neoplasms is a factor in oncogenesis and not simply a passenger. Studies conducted under this project are expected to provide some of the data required to reach a decision regarding the role of this virus in oncogenesis.

Proposed Course: Further studies will be made to determine the presence of "early" HSV-2 antigens in cervical tumors, the prevalence of antibodies to this antigen in patients, and the prognostic significance of these antibodies. Cervical tumor cell cultures will be analyzed for expression of persisting HSV-2 genome or of a fraction of a genome. A search will be made for HSV-2 specific antigens on the surface of tumor cells, biopsied, exfoliated, or cultured *in vitro*.

Date Contract Initiated: May 5, 1971

Current Annual Level: $80,000

KAROLINSKA INSTITUTE (NO1-CP3-3316)

Title: Studies on the Significance of Herpes-type Viruses and RNA Viruses in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officers (NCI): Dr. Charles W. Boone
Mr. J. T. Lewin

Objectives: To elucidate EB virus-cell-host interactions, mechanisms of cell-mediated anti-tumor immune reactions, and regulations of Type C virus expression in defined systems.

Major Findings: Part of the EBV-genome is covalently integrated with the host cell DNA in non-producer Raji cells. Another part occurs in the form of free 65S DNA, suggestive of open circular structures. Among various human lymphoproliferative malignancies, African Burkitt lymphoma (BL) was unique; 97% of histologically confirmed in vivo tumors contained EBV-DNA and the EBNA antigen. No other lymphoproliferative malignancies carried detectable genomes. Viral RNA transcripts in non-producer Raji cells may code, if translated, for 10-15 proteins. They represent a subset of the sequences found in the producer P3HR1 line. A number of new somatic cell hybrids have been produced by the fusion of pairs of lymphoblastoid cell lines, including genome positive and genome negative lines. They are used to study host control mechanisms that restrict the viral cycle. In mouse fibroblast/Burkitt lymphoma hybrids, evidence was obtained that the EBV-genome can only be associated with a minority of the human chromosomes. The EBV-determined membrane antigen (MA) could be subdivided into an early and a late component. During the viral cycle, EBNA is present in EA positive cells. Biological differences could be demonstrated between different viral isolates. P3HR1 virus was non-transforming for cord blood cells. In the genome negative BJAB line, P3HR1 virus induced EBNA, followed by EA, leads to an abortive, suicidal cycle. B95-8 and a number of other virus isolates transformed cord blood cells. In BJAB cells they induced EBNA only. In all probability, the difference between transforming and non-transforming viruses may be due to a variation in the degree of dependence on the host cell control, preventing the entry of the cells into the viral cycle. In BL and NPC patients with "discordant" antibody patterns (low MA, high EA and VCA titers), circulating complexes have been found with MA specificity. In the kidney of a BL patient, immune complexes were found with VCA specificity. In Hodgkin's disease, elevation of the EBV antibody titer is in all probability, secondary to T-cell depression. Nasopharyngeal carcinomas were propagated in nude mice, thereby purifying tumor cells from human lymphocytes (mainly T-cells) and conclusively proving by tests that EBV DNA and EBNA are associated with the carcinoma cells. An EBV-genome negative B-cell lymphoma line could be established from an American Burkitt lymphoma by nude mouse passage, followed by explantation into culture.

Cell-mediated immunity studies have revealed that T-cells of acute mononucleosis patients show strong EBV specific cytotoxicity, detectable after non-specifically cytotoxic, complement receptor positive cells have been removed.

Continued studies on mixed lymphocyte-tumor cell interactions revealed an inverted correlation between immunoglobulin coating and lymphocyte stimulation.

Studies on murine Type C virus models gave suggestive evidence of genetic recombination among different virus populations in somatic cell hybrids. Selection of an immunoresistant subline from an immunosensitive Moloney lymphoma led to the identification of a variant virus, defective in its ability to induce the Moloney-determined membrane antigen.

Significance to Biomedical Research and the Program of the Institute: Investigations under this project are directed to two areas of importance to overall Program. First, the recognition that certain herpesviruses induce neoplasms in animals and that EB virus and herpes simplex virus type 2 are associated with human neoplasms requires intensive study to provide a better understanding of the host-virus relationship for this group of agents. Data acquired under this project contributes to assessment of the role of herpesviruses in the causation of human neoplasms. Second, the analysis of the immunological responses of the host to tumor cell surface antigens provides basic information important in approaches to control of tumor development. The project is strongly oriented to human neoplasia, utilizing defined animal systems as required for progress in understanding the fundamental mechanisms involved.

Proposed Course: This project will continue without change.

Date Contract Initiated: April 9, 1968

Current Annual Level: $206,400

LIFE SCIENCES, INC. (NO1-CP3-3205)

Title: Studies on Marek's Disease as a Model for Herpesvirus Associated Oncogenesis

Contractor's Project Director: Dr. Jack Frankel

Project Officers (NCI): Dr. Gary R. Pearson
Mr. J. T. Lewin

Objectives: To determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease and the elucidation of the mechanisms of interaction between herpes and viruses in tumorigenesis using specific pathogen free avian hosts.

Major Findings: The avian leukosis virus (ALV) associated with Marek's disease herpesvirus pools (MDHV-A), that were extracted from skin of chickens with natural ALV infection, has been isolated and characterized. ALV was detected in MDHV-A pools directly examined by a radioimmunoassay. Experimental evidence indicated that this ALV (a member of Subgroup A) contributes significantly to the unique in vivo and in vitro characteristics of MDHV-A preparations. By contrast, ALV cannot be demonstrated in MDHV-B

pools produced in ALV-free chickens. In this connection, 5 different avian oncornaviruses affected the course of Marek's disease (MD) in LSI-SPF chickens when administered concurrently with MDHV-B. Enchancement of mortality was observed with the virus combinations compared with the responses of chickens inoculated with any of the viruses alone. The frequency of MDHV recovery from skin and feather follicle epithelium from chickens dually-infected with MDHV-B and Rous associated virus 1 (RAV-1), a member of ALV, Subgroup A, was significantly less than from chickens inoculated with MDHV-B only. Comparable results were obtained with chickens inoculated *in ovo* with RAV-2 (ALV, Subgroup B) and contact-exposed to MDHV. Viral enhancement and interference data from these *in vivo* experiments correlate with results obtained from studies conducted in cell cultures. Inoculation of chicken embryo fibroblast (CEF) cultures with RAV-2 and MDHV-B resulted in an enhancement of ALV titers and interference with MDHV-B focus formation.

An *in vitro* microcytotoxicity test was developed to characterize the cell-mediated immune responses of chickens infected with MDHV. In a preliminary study with this technique, a specific killing of target cells by sensitized lymphocytes from chickens with MD, as well as "blocking" and "anti-blocking" by sera, was observed. These results correlated with the degree of tumor development. Concurrent infection with ALV was shown to significantly affect the cell-mediated responses of chickens infected with MDHV. Washed lymphocytes in the presence of autologous serum from dually-infected chickens exhibiting minimal tumor development were markedly cytotoxic to MDHV-infected CEF target cells. By contrast, sensitized lymphocytes from chickens contact-exposed to MDHV for 8 weeks and showing tumor development characterized as extensive nodular lymphomatous masses were "blocked" in the presence of autologous serum.

Significance to Biomedical Research and the Program of the Institute: In comparison to the RNA tumor viruses, comparatively little is known concerning the role of herpesviruses in oncogenesis. Certain herpesviruses have been implicated in the etiology of carcinoma, lymphoma and leukemia in different species of animal and other viruses of this group have been shown to be strongly associated with neoplasia in man. This project provides opportunity to acquire information on one herpesvirus in relation to a malignant disease which may aid in understanding the role of herpesviruses in oncogenic processes in man.

Proposed Course: Studies on the interaction between MDHV and other viruses as this relates to the disease process will be continued.

Date Contract Initiated: November 1, 1968

Current Annual Level: $510,130

LITTON-BIONETICS, INC. (NO1-CP3-3211)

Title: Studies on Molecular Events Leading to Transformation by RNA Oncogenic Viruses

Contractor's Project Director: Dr. Alan Wu

Project Officers (NCI): Dr. Robert Gallo
Mr. J. T. Lewin

Objectives: To elucidate the molecular mechanisms by which RNA tumor viruses transform normal cells into malignant cells and to separate and purify cellular products presumed due to virus genome expression detected in leukemic human cells.

Major Findings: Molecular Biology of Human Leukemic Cells: The cytoplasmic particles isolated from human leukemic cells have some of the properties of RNA tumor virus. They are banded at a density between 1.16 to 1.19 gm/ml and contain both virus-related nucleic acid and reverse transcriptase. [^{3}H]DNA products synthesized by an endogenous reaction of the cytoplasmic particles from leukemic cells can hybridize to RNA purified from animal RNA tumor viruses; those obtained from AML and CML in acute phase hybridize best to RNA from MuSV$_K$(NRK) and those from CML to RNA from SiSV(NRK). DNA products synthesized from pellet DNA polymerase activity from phytohemagglutinin-stimulated lymphocytes fail to hybridize to 70S RNA from SiSV(NRK) and Mason-Pfizer virus (grown in NC37 cells). The reverse transcriptase from human leukemic cells was shown to contain two forms with respect to their molecular weights (70,000 and 150,000). The high molecular weight form exists in the low salt condition, while the low molecular weight form is in high salt condition. These two forms are interconvertible and have different biochemical characteristics. This change of enzyme characteristics following aggregation of enzyme molecules might have some regulatory function in reverse transcriptase activity.

Molecular Biology of Life Cycle of RNA Tumor Virus. Stimulation of the Type-C virus production by dexamethasone and inhibition by interferon were previously shown to be a post-transcriptional effect on the gene expression of RNA tumor viruses. The amount of intracellular gs protein was not significantly altered following treatment of the virus-producing cells with dexamethasone and interferon. This observation presents the possibility that dexamethasone and interferon act at the step after synthesis of viral gs proteins, such as assembly or release of viral particles. Using molecular hybridization techniques, it was observed that there is no significant increase of sarcoma-specific RNA in non-producer K-BALB cells following IdU treatment, which induces production of sarcoma viruses. Leukemia virus-specific RNA increases following induction of BALB cells by IdU. One interpretation of this finding is that some products of gene expression of endogenous leukemia virus are required for a completion of sarcoma gene expression. RNA-directed DNA polymerase is found in intracisternal Type A particles from N18 murine neuroblastoma cells. The RNA of the particles are not closely related to RNA from known murine Type C particles. This finding is currently extended to another clone of neuroblastoma N4 cells and to PHA-stimulated human lymphocytes. It is interesting to point out that the DNA product from Type A particles of human PHA-stimulated lymphocytes are somewhat related to RNA from those isolated from N18 and MOPC cells.

Significance to Biomedical Research and the Program of the Institute: The knowledge acquired is applied to the determination of the etiological relationship of viruses to leukemia in humans, to the development of diagnostic and prognostic modalities for human cancer, and ultimately, to the development of more effective control measures.

Proposed Course: Studies will continue to further define the nature of the virus-like activity expressed in human leukemic cells.

Date Contract Initiated: September 1, 1972

Current Annual Level: $423,060

LITTON-BIONETICS, INC. (NO1-CP5-3509)

Title: Investigations of Suspected Oncogenic Viruses in Nonhuman Primates

Contractor's Project Director: Dr. Harvey Rabin

Project Officers (NCI): Dr. Gary Pearson
Mr. J. T. Lewin

Objectives: To investigate host-virus relationships established by viruses known or suspected to induce neoplasia in animals or humans using nonhuman primates of different species and to determine host immune responses following virus infection correlating these with viral reproduction and pathological processes.

Major Findings: Herpesvirus saimiri. Studies continued on the mechanism involved in the loss of lymphocyte competency which precedes the onset of disease in infected owl monkeys. It has been found that incompetent lymphocytes confer a loss of PHA response to normal lymphocytes in vitro. Studies also proceeded on extracts of the HVS-tumor-derived lymphoblastoid cell line, MLC-1. Extracts of these cells are toxic for normal lymphocytes of several primate species, and for some, but not all, cells of established lymphoblastoid lines, but not for epithelioid cells of line Vero. The extracts however, significantly inhibit herpesvirus plaque formation in treated Vero cells. Physical characterization of the active factor(s) in the extract is in progress. Serologically, collaborative studies have shown that a lymphocyte dependent cytotoxic antibody arises in infected owl monkeys and apparently shows a disease related pattern. Prednisolone treatment of a chronically infected capuchin monkey led to loss of antibody to HVS-associated antigens but not to potentiation of disease.

Epstein-Barr Virus. Studies have shown that both owl monkey and marmoset lymphocytes show cytotoxic activity in vitro in ^{51}Cr-release assays. However, lymphocytes from control monkeys also exhibit such activity. Serologically, evidence has been found for antibody to EBV-associated antigens in the sera of some control owl monkeys in their colony.

Oncornavirus. A strain of Type C virus which is cross-reactive with feline sarcoma virus has produced tumors in 5 young adult white-lipped marmosets.

The tumors were highly undifferentiated and had brief latent periods. All inoculated marmosets seroconverted to anti-gs antibody. Inoculation of this virus into marmosets previously injected with EBV resulted in a tumor in one monkey. No potentiation of EBV-related disease was noted, however.

Foamyviruses from Russian baboons. Both strains of foamyvirus were shown to cross-react with antisera to type 1 foamyvirus but not with antisera to types 2 through 7. Electron microscopy has shown typical immature particles. No hemagglutinin has been demonstrated in the virus preparations.

Significance to Biomedical Research and the Program of the Institute: Certain herpesviruses have been shown to be associated with some human neoplasms. The study of neoplastic diseases induced in experimental animals by infection with herpesviruses is expected to contribute to a better understanding of the nature of the virus-cancer associations that exist in humans.

Proposed Course: Two areas require attention: the determination whether RNA viruses are involved in the oncogenic responses to herpesvirus infections and the determination of the influence of host immune responses to virus-induced antigens on the development of neoplasia.

Date Contract Initiated: September 1, 1974

Current Annual Level: $304,900

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP3-3366)

Title: Characterization of Nucleic Acids of the Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Paul C. Zamecnik

Project Officers (NCI): Dr. George Vande Woude
Mr. J. T. Lewin

Objectives: The primary objective is the determination of the possible molecular mechanisms by which oncogenic viruses change the metabolism of susceptible host cells. In order to define this system, long range biochemical studies on the RNAs of AMV have been initiated and involve the chemical characterization of the nucleotide structure of viral RNAs and biochemical functions of the viral RNA fractions as measured by their activity in conjunction with RT from the virus and the ability of the 4S RNA fractions to accept amino acids.

Major Findings: Selective digestion of the 35S RNA of avian myeloblastosis virus (AMV) by specific enzymes, followed by isolation of poly(A)-containing fragments has enabled selective isolation of nucleotides adjacent to the poly(A) region along with the poly(A). Current studies involve the use of ribonuclease A and ribonuclease T_1. Ribonuclease A in high salt concentrations hydrolyzes nucleic acid sequences at U and C residues and yields runs of A-3'OH with possibly some G and A nucleotides internal to the poly(A) but

no C or U. On the other hand, ribonuclease T_1 (which splits at G residues) yields the terminal poly(A) plus internal U, C and A but no G. Such poly(A)-containing fragments have been isolated and their size and composition are being determined by periodate oxidation-H^3-borohydride reduction technique both on terminal and total nucleotides. Preliminary results following treatment with ribonuclease T_1 indicate that just interior to the 3' poly(A) there is a U and a C residue. The possibility of an A residue was not ruled out. Results also indicate that a slight amount of G could also be in that segment of the molecule. Results with ribonuclease A indicate that there is a small but significant amount of G adjacent to the poly(A) sequence and that also a small amount of C and U may be present.

The presence of a polyadenylic acid sequence at the 3'-hydroxyl terminus of AMV 35S RNA has provided a convenient starting point for sequence analysis of this RNA molecule by enzymatic copying, using oligo thymidylic acid as primer to initiate synthesis of DNA complementary to viral RNA. Initial experiments using DNA polymerase I from E. coli to "reverse transcribe" AMV RNA were compromised by extensive homopolymer synthesis. More recent studies using reverse transcriptase enzyme from AMV, however, have proved more fruitful. The results indicate that copying of AMV 35S RNA by reverse transcriptase is a highly specific process dependent on the presence of oligo thymidylic acid primer to initiate copying presumably on the poly(A) sequence, and that the 35S RNA preparation used in these studies is free of endogenous primer molecules. The lack of incorporation of radioactivity in the absence of unlabelled dTTP can be the result of either greatly reduced priming efficiency of oligo pT on the poly(A), or more likely the presence of one or more A residues in the RNA template interior to the poly(A) sequence but preceding the first G resi[illegible] (α-^{32}P dCTP incorporation) or preceding the first U residue (α-^{32}P [illegible] incorporation). Incorporation of α-^{32}P dATP in the absence of dCTP strongl[illegible] suggests that the first G residue in the RNA template is interior to the [illegible]irst U residue. The preliminary results suggest that AMV 35S RNA is a ho[illegible]ogeneous population of molecules with respect to the first nucleotide residue adjacent to the poly(A) sequence since only dGTP is incorporated in the absence of other deoxytriphosphates. Whether all 35S molecules are identical in sequence awaits more extensive sequence analysis.

A sensitive chemical procedure has been developed which introduces high specific activity ^{32}P-label at phosphorylated 5'-ends of low and high molecular weight RNAs. The method is based on the reaction of [^{32}P] diimidazolidate of orthophosphate which reacts exclusively with primary phosphates. The internucleoside phosphate diesters are weak nucleophiles and remain unaltered. The high specific activity ^{32}P-labeled ribonucleic acid can then be analyzed for 5'-end group or 5'-terminal oligonucleotide sequences. The chemical procedure enables the isolation of predominantly intact ^{32}P-labeled 35S from the 70S RNA of avian myeloblastosis virus.

It was recently observed that tumor formation occurred at the site of inoculation of 25 immunologically impaired nude mice, injected with tissue culture cells from four spleens grown from four separate patients with Hodgkin's disease. Chromosome analysis of tissue cultures grown from the tumors induced by the human material reveals that the tumors are human and

and not mouse. Murine fibroblastic tissue does, however, weave its way among the pavement type, frequently multinucleated cells of the in vivo tumors produced in the nude mouse.

Significance to Biomedical Research and the Program of the Institute: This project was initiated in the expectation that elucidation of the nucleotide sequences of the 70S RNA of AMV and possibly other oncogenic RNA viruses might reveal segments active in specific functions and as binding sites of viral polymerases or inhibitors, thereby increasing our knowledge of transcription processes.

Proposed Course: Studies are expected to continue on the analyses and the acromolecular sequencing of large molecular weight RNA of AMV.

Date Contract Initiated: June 29, 1971

Current Annual Level: $139,870

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP4-3222)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-immunologic Methods

Contractor's Project Director: Dr. Paul H. Black

Project Officers (NCI): Dr. Michael A. Chirigos
Mr. J. T. Lewin

Objectives: To determine the relationships between chronic allogeneic disease, immunosuppression, and interferon inducers on the activation of covert infections by oncogenic RNA viruses.

Major Findings: Various immunosuppressive protocols differed in their ability to prolong DBA/2 skin grafts on BALB/c mice. The normal median survival time (MST) of DBA/2 skin on BALB/c mice was 14.8 ± 2.1 days. Seventy-five percent of ALS-treated mice had surviving grafts when sacrificed at day 28. The MST with high-dose cyclophosphamide was 20.8 ± 5.8 days, although 45% of the mice in this group still had surviving grafts on day 28. The MST with low-dose cyclophosphamide was 16.0 ± 5.6 days. Neither high- nor low-does cortisone was effective in prolonging graft survival significantly, the MST's being 15.9 ± 4.1 and 15.1 ± 1.8, respectively. The largest number of virus-positive animals was found in the two groups where graft survival was significantly prolonged. Of the grafted, ALS-treated mice (Group B), 65% were virus-positive, whereas in the grafted, high-dose cyclophosphamide group (c), 69% were positive. None of the other groups had significant degrees of virus activation. In both the graft plus ALS and graft plus cyclophosphamide groups, both mouse-tropic and xenotropic viruses were activated. Detection of Type C virus activation following immunostimulation reactions may depend on persistent immunosuppression following the induction event. Thus, although lymphocyte responses against histocompatibility antigens are sufficient to induce Type C viruses, continued replication of these agents may depend on an ineffective mechanism for virus elimination. It is

likely that the development of neoplasia in such situations is also dependent on a persistent hyporesponsive state to virus-associated tumor antigens. Additionally, both mouse-tropic and xenotropic Type C viruses can be activated during skin graft rejection reactions. In some animals, both types were activated; in others, one or the other. Previous studies have indicated that other forms of immunological reactions, i.e., graft versus host reactions, could also result in the activation of both mouse-tropic and xenotropic viruses.

The studies showed that (BALB/c X A/J)F_1 mice can develop cell-mediated responses against antigens on syngeneic cells infected with endogenous mouse-tropic C-viruses. It appears that at least some strains of mice may be exposed to antigens associated with Type C viruses at an early age, prior to detectability of infectious virus in these animals, and that they can respond with both humoral and cell-mediated immune responses against these antigens. Variations in the production of these immune responses may markedly influence the likelihood of Type C virus replication and subsequent oncogenesis. The interactions among endogenous Type C viruses, host immune responses and neoplasia are complex. The unraveling of these complexities will require longitudinal studies in various mouse strains of specific humoral and cell-mediated responses to both viral and tumor antigens. Understanding these relationships may well provide a rational basis for eventual vaccination programs against endogenous oncogenic viruses.

Significance to Biomedical Research and the Program of the Institute: This project contributes new information concerning immunological factors involved in the activation of covert virus and its influence on the development of virally-induced cancer.

Proposed Course: The present studies on the interactions of immunostimulation, immunosuppression, and leukemia virus activation in the murine model systems will be extended. Studies are planned to expand these observations to include similar situations in man.

Date Contract Initiated: September 15, 1971

Current Annual Level: $121,370

MELOY LABORATORIES (NO1-CP5-3508)

Title: Cell Biology Facility: Mechanisms of the Immune Response to Squamous Cell Carcinoma, Adenocarcinoma, and Fibrosarcoma in the Mouse and Experimental Immunotherapy

Contractor's Project Director: Dr. Ronald Gillette

Project Officers (NCI): Dr. Charles W. Boone
Mr. J. T. Lewin

Objectives: To elucidate the mechanisms of specific tolerance to tumor exhibited by the cellular immune system in a tumor-bearing animal, to develop improved in vitro and in vivo assays for detecting tumor specific antigens and antibodies, and to develop systems of immunotherapy.

Major Findings: Reagents have been prepared for testing the virus augmentation of tumor transplantation antigen (TTA) effect in humans. Three human melanoma lines and one normal human fibroblast line, both virus-infected and non-infected, have been prepared for skin testing of patients with malignant melanoma at Roswell Park Memorial Institute.

Experiments have been performed in animals to determine the most effective injection schedule of virus-infected tumor homogenates that maximizes the effect of virus augmentation of TTA. Thus far, injections of homogenate given at weekly intervals for three weeks appears inferior to smaller numbers of injections at closer intervals. Freund's complete adjuvant was found to be ineffective in this system. The adoptive radioisotopic footpad assay has been developed for testing the immune responsiveness of different lymphoid cells.

Plasma membranes have been purified on Sepharose columns in an attempt to purify the virus antigen-TTA complex. In the development of affinity chromatography for membrane purification, it was found that plasma membranes bind extensively to Con A-Sepharose columns, but these membranes are difficult to elute. The paired label radioimmunoassay had been adapted to monitor the time course of vesicular stomatitis virus (VSV) infection of cells. The results indicate that shorter infection times yield more viral cell surface antigen.

Methods for collagenase disaggregation of solid tumors have been developed, and the tumorigenicities of the subsequent cell suspensions are now being tested. A new and improved assay for testing the cell immune response in tumor immunity has been developed. This method supplements the standard ^{51}Cr release assay and probably detects a broader range of immune cell response to tumor antigens.

Significance to Biomedical Research and the Program of the Institute: Prior infection of tumor cells with influenza virus to augment the immunogenicity of specific tumor transplantation antigens obtained in cell-free fractions of tumor cell homogenates provides a method whereby a patient could be immunized without the risk associated with X-irradiated viable cells. Research on the mechanisms of the immune response to tumor could provide methods to benefit the cancer patient both in diagnosis and in therapy.

Proposed Course: The investigations on the relationship of cell surface structure to the host's response to tumor cell antigens will be continued.

Date Contract Initiated: August 20, 1971

Current Annual Level: $346,420

MELOY LABORATORIES (NO1-NS2-2306)

Title: Oncogenic Potential of Herpesviruses in Primates

Contractor's Project Director: Dr. John Verna

Project Officers (NCI): Mr. J. T. Lewin
(NINDS): Dr. Amos Palmer
Dr. W. T. London

Objectives: To evaluate herpes simplex virus type 2 (HSV-2) as a potential agent of cervical carcinoma in Cebus monkeys.

Major Findings: This is a collaborative project between NCI and NINDS. The colony of Cebus monkeys for this project has reached the required size at 467 animals. A total of 173 animals have received virus inoculations and 67 have received control inoculations. Virological, cytopathological and immunological monitoring of these animals is accomplished at Emory University under contract NO1-NS2-2301. An infection rate of more than 80% has been obtained with virus injected intradermally into the cervical epithelium. Minor cytological changes were noted in 2 control and 30 virus-inoculated animals. Abnormal diagnoses were observed in 23 virus-inoculated animals. Six of these monkeys have had more than 4 abnormal smears. These have persisted for from 10 to 28 months with apparent progressive changes in 4 of the animals. Colposcopic examinations agree with cytological findings in 77% of the cases.

Significance to Biomedical Research and the Program of the Institute: HSV-2 has been shown to be associated with invasive cervical carcinoma in humans. This project was initiated to determine whether repeated infections in a nonhuman primate that responds to infection by this virus in a manner similar to humans would result in cervical malignancy. The data acquired in this and related projects is expected to provide a better understanding of the nature of the relationship observed between this virus and the cancer.

Proposed Course: This project is expected to continue for at least 3 more years.

Date Contract Initiated: March 30, 1972

Current Annual Level: $264,277

MERCK AND COMPANY, INC. (NO1-CP1-2059)

Title: Research on Oncogenic and Potentially Oncogenic Viruses, Virus Production and Vaccine Development

Contractor's Project Directors: Dr. Maurice Hilleman
Dr. Vivian M. Larson

Project Officers (NCI): Dr. Michael A. Chirigos
Mr. J. T. Lewin

Objectives: To conduct investigations designed to develop vaccines or other agents for the prophylaxis and therapy for human neoplasia of suspected viral etiology.

Major Findings: This contract to evaluate means for immunologic control of cancer includes three distinct, but complimentary, projects: (1) evolvement of feline leukemia-sarcoma virus vaccines, (2) evolvement of Herpes simplex type 2 virus (HSV-2) vaccines, and (3) evolvement of tumor cell antigen vaccines. A significant portion of this year's program effort was concerned with initial phases of vaccine development such as optimal propagation conditions for candidate vaccine viruses, standardization of assay techniques, establishment of vaccine animal test models and study of host immune responses.

Exploratory vaccine studies were conducted with flow zonal purified Kawakami-Theilen feline leukemia virus (KT-FLV). The virus is inactivated at 96 hr. with 1:4000 formalin at 4°C. Potency tests in guinea pigs showed that such preparations were antigenic; virus neutralizing and cytotoxic antibody induction was demonstrated. Preliminary tests in specific pathogen-free (SPF) cats indicated that such vaccines induced significant levels of cytotoxic antibody but little or no virus neutralizing antibody. When such cats were challenged with Snyder-Theilen feline sarcoma virus the induction of tumors was the same as in control cats. This probably represents inadequate homologous immunogenicity and monovalent serotype vaccines are being pursued.

Improvements in large scale HSV-2 production resulted in a 20-fold increase in yield in primary chick embryo (CE) cell cultures. Infected CE cells were disrupted by sonication and fractionated by Sephadex and calcium phosphate (Brushite) chromatography and by extraction with solvents. The fractions were examined for glycoproteins and are being tested for biological activity. At least 18 virus-directed glycoproteins were detected in HSV-2-infected CE cells in radioactive labeling experiments.

Soluble adenovirus type 12 (Adv 12) tumor cell antigens were prepared and tested for protective efficacy against tumor growth. Hamster Adv 12 tumor vaccines prepared by Hollinshead's procedure failed to protect hamsters in repeated experiments. Adv 12 - CBA mouse tumor vaccines prepared by the method of Potter and coworkers were shown to protect CBA mice against tumors in repeated experiments. This crude soluble vaccine was fractionated by Sephadex chromatography and at least eight glycoproteins were detected in the fractions.

Significance to Biomedical Research and the Program of the Institute: If viruses are proven to be essential for the development of some human cancers, a vaccine may effectively prevent or minimize infection or pre-sensitize the host to reject antigens expressed during the earliest stages of tumor development. This project was initiated to determine whether vaccines acceptable for use in humans were of practical value for these purposes.

Proposed Course: The contractor is concentrating solely on the evaluation of the effectiveness in SPF cats of vaccine preparations from individual serotypes of feline leukemia viruses.

Date Contract Initiated: March 1, 1971

Current Annual Level: $800,000

UNIVERSITY OF NAPLES (NO1-CP3-3314)

Title: Studies of Non-Virion Antigens of Herpes Simplex Viruses

Contractor's Project Director: Dr. Giulio Tarro

Project Officers (NCI): Dr. Charles Boone
Mr. J. T. Lewin

Objectives: The determination of the presence of non-virion antigens induced by herpes simplex virus in tumors of the urogenital tract and of antibody to non-virion antigens in the sera of cancer patients in order to obtain added information on the relationship between herpesvirus type 2 and tumors of the genitourinary system.

Major Findings: Data are reported on the multiplicity of herpes simplex virus (HSV) nonstructural antigens in guinea pig kidney (GPK) and rabbit kidney (RK) cells infected with the same strain of virus. Both the HSV types 1 and 2 non-virion materials consist of more than one component for which the immunized guinea pigs produce distinct antibodies. It was possible to separate by polyacrylamide gel electrophoresis (PAGE) HSV-induced non-virion antigen markers not only from cells undergoing lytic infection by the virus, but also from viable cells from squamous cell carcinomas of head and neck and urogenital tract. HSV non-virion antigens were detected in selected cancers by CF test after trypsinization of fresh surgical specimens (lip, cervix uteri, kidney) and by anticomplement immunofluorescence (lip, larynx). The finding of HSV non-virion antigens in selected tumors as expression of a repressed viral genome proves a continuing release of virus-specific messages and would support an important role of the virus in the development of the tumor. Cell-mediated immune responses to herpes simplex virus type 1 or type 2 virion and non-virion antigens were assessed in control subjects and in patients with oral leukoplakia, carcinoma or recurrent herpes labialis. Enhanced transformation was found in lymphocytes from patients with recurrent herpes labialis or with leukoplakia showing epithelial atypia and depressed responses were found in carcinoma. These changes were not seen when vaccinia antigens were used. Very significant positive correlations were found between the responses to each of the herpesvirus antigens and there was no evidence for a specific response to the non-virion antigen in any group of patients. Lymphocytes from patients with recurrent herpes labialis required higher concentrations of herpes antigen for optimal stimulation than lymphocytes from other patients. Lymphocyte responses were not diminished when a herpes virion-non-virion preparation was stored at 4°C to inactivate the non-virion antigen. A specific increase in cell mediated immunity to herpes virus in epithelial atypia was confirmed, but further purification of the non-virion antigen is necessary before specific cell-mediated immune responses to this antigen can be assessed.

Significance to Biomedical Research and the Program of the Institute: Past studies suggested that patients with cervical cancer and other genitourinary cancers responded immunologically to a herpes simplex virus-coded "non-virion" antigen. This project was continued for a final year to complete the serological studies underway to further evaluate the observations previously reported.

Proposed Course: This project was terminated on April 8, 1975.

Date Contract Initiated: April 9, 1971

Final Annual Level: $40,000

UNIVERSITY OF NORTH CAROLINA (NO1-CP3-3336)

Title: Molecular Studies on Herpes-type Viruses of Potential Oncogenicity

Contractor's Project Director: Dr. Joseph Pagano

Project Officers (NCI): Dr. Berge Hampar
Mr. J. T. Lewin

Objectives: To define at the molecular level the virus-cell relationships for herpes simplex virus type 2 (HSV-2) and human cervical carcinoma and for Epstein-Barr virus (EBV) and Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC).

Major Findings: A portion of the EBV genome extracted from virus derived from HR1K cells (prototype virus) might be deleted in nasopharyngeal carcinomas from Singapore. These observations were extended and confirmed with additional analyses conducted on other specimens from different geographic locations. As much as 25% of the prototype EBV genome may be missing in tumor tissue. It is not known whether these results indicate simple deletions with retention of a defective genome or whether there is replacement of the missing sequences or genome segments by heterologous sequences in a strain of EBV different from the prototype virus. A specimen of American Burkitt's lymphoma that was tested does not contain detectable EBV DNA by DNA-DNA renaturation kinetics analyses.

HSV-2 DNA was fractionated with the restriction endonuclease endo EcoRI into a convenient number of fragments (less than 20). A technique for in vitro iodination of herpesvirus DNA was developed. Ten specimens of cervical carcinoma were examined for the presence of HSV-2 DNA, and only one of these showed any indication of the viral genome. This specimen was from Taiwan and was negative for infectious HSV (Russell Alexander) but positive for infectious cytomegalovirus. There is less than 5% homology between HSV-2 DNA and cytomegalovirus, so exactly what these results indicate at the present time is unclear. Two problems exist: The virus strain used may not have that DNA segment(s) which (presumably) exists in the cervical tumors probed. The second problem is that the assay may not be sensitive enough to detect the presence of a small portion of the HSV-2 genome. A fresh genital virus isolate

from the geographic region of the biopsy specimens will be obtained. This may contain sequences common to those presumed to be in the tumor. To increase the sensitivity of the test procedure, HSV-2 DNA is being prepared in vitro with a specific activity of 10^8 cpm/μg -- a 20-100 fold increase over previous preparations.

The cytotoxic effect of leukocytes from patients with infectious mononucleosis for EBV-transformed lymphoid cell lines was assessed. Cytotoxicity is measured by a Cr release assay in which 10^4 target cells are incubated with peripheral blood leukocytes for 18 hours at 37°. There is a basic level of cytotoxicity among normal donors for Raji cells. Peripheral blood leukocytes taken from patients with EBV-associated mononucleosis during the first two weeks of overt disease were significantly more cytotoxic than those of normal donors. This increase in cytotoxicity begins to disappear as the disease progresses.

Tests on 2 pellets of cells obtained from patients with chronic lymphatic leukemia (CLL) by plasmapheresis failed to detect EBV DNA directly in the lymphocytes either by cRNA-DNA hybridization or by DNA-DNA renaturation kinetics analysis. A pellet of cells obtained directly from the peripheral blood of a patient with acute lymphocytic (T-cell) leukemia also was negative in test for the EBV genome. Cytomegalovirus (CMV) undergoes an abortive infection in human lymphoblastic cell lines with CMV viral DNA synthesis taking place only in B-cell lines bearing the EBV genome. It is not known whether any virus-specified proteins are induced. This is a phenomenon of potential significance in the pathogenesis and reactivation of CMV infection.

Extensive studies in which HR1K cells, Raji cells, and Raji cells superinfected with EBV, as well as a number of other B and T cell lines have been assayed for the possible presence of an EBV-specified or induced thymidine kinase have met with negative results.

Significance to Biomedical Research and the Program of the Institute: The studies accomplished verify the close relationship between EB virus and cells in BL biopsies. The continuing refinement by the contractor of the molecular methods provide useful tools in the study of viral associations with tumor cells where repression of virus genetic expression masks the presence of infection.

Proposed Course: This project will be completed during the next year.

Date Contract Initiated: April 25, 1972

Current Annual Level: $131,730

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CP4-3217)

Title: Biohazards Control and Containment in Oncogenic Virus Research

Contractor's Project Director: Dr. Richard G. Olsen

Project Officers (NCI): Dr. Alfred Hellman
Mr. J. T. Lewin

Objectives: To evaluate the prophylactic vaccination of cats against RNA tumor virus oncogenesis.

Major Findings: Past studies had shown that homogenates of tumors induced by ST-feline sarcoma virus, after inactivation of the virus, provided effective protection to cats against sarcoma-virus challenge. Queens that carry serum neutralizing (SN) antibody to feline sarcoma virus induced by a UV-inactivated virus vaccine preparation do transmit to their offspring a passive immunity to sarcoma virus challenge. Satisfactory protection was not induced in kittens by active immunization. Although a sarcoma virus vaccine may elicit production of specific SN antibodies to the virus, no antibody is produced against the feline oncornavirus cell membrane antigens (FOCMA). A rise in FOCMA antibodies in cats after challenge with feline sarcoma virus appears to correlate with the development of tumor growth. The production of FOCMA is to be accomplished using the FL-74 cell line infected with the KT-feline leukemia virus. A cytotoxicity assay has been developed as a means to monitor the purification of FOCMA for use in trials for protection of animals against virus challenge.

Significance to Biomedical Research and the Program of the Institute: The trials to determine the effectiveness of vaccination in the cat as a protective measure against oncornavirus-induced neoplasia have a two-fold purpose. The results provide information concerning the nature of the immune response providing the best degree of protection against infection or ensuing disease in a genetically heterogeneous population of animals susceptible to certain oncornaviruses. Secondly, knowledge is obtained of the nature of the vaccine preparation providing protection to the actively immunized subject and secondarily to newborn progeny highly susceptible to oncogenic viruses.

Proposed Course: The project will be continued to determine the value of purified FOCMA in protection of vaccinated animals. The cellular immune responses, as well as humoral responses to vaccines will be evaluated.

Date Contract Initiated: June 25, 1965

Current Annual Level: $152,280

PENNSYLVANIA STATE UNIVERSITY (NO1-CP5-3516)

Title: Studies on the Oncogenic Potential of Defective Human Viruses

Contractor's Project Director: Dr. Fred Rapp

Project Officers (NCI): Dr. Peter Fischinger
Mr. J. T. Lewin

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: A quantitative focus-forming assay using 3T3 cells has been refined for the demonstration of the cell-transforming potential of herpes simplex viruses; current tests suggest it may also prove useful for cytomegalovirus. Hamster cells previously transformed by photo-inactivated virus were oncogenic when transplanted to the same species. The original cells, as well as cells from the tumors, continue to synthesize virus-specific antigens localized in the cytoplasm in a proportion of the cells. Micro-cytotoxicity tests have revealed specific antigens at the surface of herpes simplex and cytomegalovirus transformed cells detectable with spleen cells from animals bearing tumors or immunized with the corresponding virus.

A relationship between induction of host DNA synthesis and replication of cytomegalovirus was established. The virus was shown to induce host DNA and to replicate in lymphocytes from immune (neutralizing antibody) donors, but did neither in lymphocytes from non-immune donors. Present experiments are designed to determine whether this virus can transform cells from various donors.

Attempts to induce Epstein-Barr virus in a variety of cells with X-rays, ultraviolet light, and acridine orange failed. The results support the hypothesis that the association of virus DNA with host DNA in the systems tested differs from that in lysogenic bacteria. Continued characterization of an inhibitor of virus replication revealed that it is specific for EBV and CMV, but does not interfere with herpes simplex viruses. The inhibitor requires poly-ornithine for activity and does not appear to be interferon. Its role in maintenance of the transformed state due to EBV is under investigation.

Significance to Biomedical Research and the Program of the Institute: The observations made under this project indicate that some strains of herpesviruses commonly afflicting humans may have oncogenic properties which may be expressed under certain conditions. Better assay procedures for transforming potential may provide a means to assess the significance of virus strain differences within populations leading to a better understanding of the nature of the association of HSV-2 with human cancer.

Proposed Course: Investigations on herpesviruses in relation to cancer will continue.

Date Contract Initiated: October 27, 1969

Current Annual Level: $493,000

ROCKEFELLER UNIVERSITY (NO1-CP3-3306)

Title: Evaluation of Methods for Isolation of Virus from Human Neoplasms

Contractor's Project Director: Dr. Hidesaburo Hanafusa

Project Officers (NCI): Dr. Charles Boone
Mr. J. T. Lewin

Major Findings: Chemical and physical tests failed to yield evidence of the presence of Type C virus in cultures originated from human sarcoma tissue. Treatment of these cultures with halogenated pyrimidines did not induce the release of virus particles. Co-cultivation with other cells combined with infection by viruses that could possibly provide "helper" functions also was without success.

Cell lines originated from 3 sarcoma specimens grew relatively well in continuous culture. A number of methods have been applied to establish the malignant identity of these cells.

Significance to Biomedical Research and the Program of the Institute: This study demonstrated the difficulties in identifying sarcomatous cells in culture and showed that the methods evaluated could not detect Type C virus activity in cells cultured from human sarcomas.

Proposed Course: This project was terminated April 26, 1975.

Date Contract Initiated: April 27, 1971

Final Annual Level: $121,000

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP3-3219)

Title: Studies of Tumor Viruses in Small Primates

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Michael Chirigos
Mr. J. T. Lewin

Objectives: To study selected viruses and virus-induced neoplasia in marmosets.

Major Findings: Tests were initiated to determine the susceptibility and response of marmosets to infection by endogenous Type C viruses recovered from baboons. Lymphoma induction with the B95-8 strain of EBV in cotton-topped marmosets was confirmed. The common marmoset is equally susceptible to tumor-induction by this EBV strain. A strain of Herpesvirus saimiri grown at 39C lost its ability to induce lymphoma in marmosets. This loss of oncogenicity was stable through 3 serial marmoset passages. The ability of Herpesvirus ateles to transform primate lymphoid cells in vitro was confirmed. Herpes simplex virus type 2 caused transformation of rat and of marmoset cells at elevated temperature. The rat transformants were oncogenic in vivo. Tests on the marmoset cells are not complete.

Significance to Biomedical Research and the Program of the Institute: Restrictions in the host range of viruses require the availability of a primate animal for the study of viruses associated with neoplasia in humans. The marmoset has proven to be most responsive to the oncogenic activity of known tumor viruses. This species is particularly valuable for the study of

herpesvirus strains that induce malignant transformation. Viruses of the herpes group are widely distributed among animal species and infections by a number of these agents are known to result in the development of malignant tumors in the host. It is most important to expand our knowledge of herpesviruses with respect to cancer in primate animals and of their potential for oncogenicity in humans.

Proposed Course: This project will be continued with the following immediate goals: SSV-1/SSAV-1 and endogenous primate Type C virus nucleic acids and antigens will be used as probes for detection of human and other primate oncornaviruses. Attempts will be made to isolate human and additional primate oncornaviruses. Marmosets infected with potentially oncogenic human viruses will be observed for tumor development. Studies of Type C RNA virus-induced gliomas in marmosets will be continued. Additional studies will be made of the natural history, pathogenicity, virology and immunology of oncogenic primate herpesviruses and with detailed characterization of the various isolates. The immune responses of tumor-bearing primates will be followed.

Date Contract Initiated: March 15, 1962

Current Annual Level: $600,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP4-3214)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. R. L. Heberling

Project Officers (NCI): Dr. Alfred Hellman
Mr. J. T. Lewin

Objectives: To study the viral flora in placentas and embryos of primates with emphasis on Type C viruses.

Major Findings: The endogenous Type C viruses detected in the placentas of baboons were studied. These viruses were inoculated into different species of animals, but, as yet, no overt expressions of virus activity have been observed. Electron microscope observations demonstrated the presence of virions in the perivitteline space and zona pellucida of the follicular oocyte, thus providing direct evidence for vertical transmission of these viruses. Additional virus isolations made from animal placentas and embryonic tissues gave added evidence for the universal occurrence of genetic information for Type C viruses in cells of diverse origins.

Significance to Biomedical Research and the Program of the Institute: Evidence was provided for the existence in "normal" primate tissue of vertically transmitted Type C viruses. Further study is required to determine whether these agents have any direct relationship to the development of malignancy in any primate.

Proposed Course: Efforts will be continued to determine the function of these virus particulates in the primate host.

Date Contract Initiated: June 3, 1971

Current Annual Level: $279,320

UNIVERSITY OF TEXAS (NO1-CP3-3304)

Title: Studies on the Relationships of Viruses to Human Neoplasia

Contractor's Project Director: Dr. Leon Dmochowski

Project Officers (NCI): Dr. Gary Pearson
Mr. J. T. Lewin

Objectives: To pursue a systematic study of neoplastic disease in selected human patients to determine whether an association of a virus with these cancers may exist.

Major Findings: During the terminal period of this project, efforts were concentrated on completion of the studies under way. The work included the biochemical characterization of high molecular weight RNA released from some cultured human cells originated from tumor tissue. Attention was given to the eradication of mycoplasma from cultures. Two cell lines were established from breast carcinoma tissue. The sera from patients with osteosarcoma were observed to react with partially purified antigens of the tumor when tested by the immunodiffusion method in gels.

Significance to Biomedical Research and the Program of the Institute: The principal effort under this project was to determine whether a virus could be isolated from human neoplasms by existing or newly devised methods. One virus recovered from a lymphoma patient was subjected to intensive study. Biological, serological and molecular methods yielded evidence suggestive of the expression of viral information in tumor tissue thereby confirming similar observations in other laboratories.

Proposed Course: This project was terminated on October 31, 1974.

Date Contract Initiated: March 19, 1965

Final Annual Level: $369,570

SUMMARY REPORT

3. IMMUNOLOGY-EPIDEMIOLOGY SEGMENT

July 1, 1974 to June 30, 1975

During this fiscal year, the Immunology-Epidemiology Segment continued to concentrate on the role of herpesviruses in cancer, adding two complementary contracts to develop assays of cell-mediated immunity (CMI) to herpes simplex type 2 (HS-2) to the program. As a result of two RFP's, five new contracts (including the two herpes CMI contracts) brought the total number of I-E Segment contracts operating throughout the entire fiscal year to 24; an additional contract was transferred to the I-E Segment in the second half of the fiscal year. Completion of workscope and/or the decreased emphasis on tumor immunology due to the establishment of the Tumor Immunology Program in the Division of Cancer Biology and Diagnosis led to the phasing out of five I-E Segment contracts.

Ten I-E Segment contracts participated in studies on the role of viruses (particularly herpesviruses) in human lymphoma. Under the supervision of the International Agency for Research on Cancer (IARC), the prospective study for evaluating immunity to EBV prior to and subsequent to the onset of Burkitt's lymphoma indicated that BL is not the result of a primary infection with EBV in a child who managed to escape EBV infection in very early life. Sera collected by the IARC project and tested at Children's Hospital of Philadelphia indicated that antibodies to the viral capsid antigen (VCA) were present up to a year prior to the onset of BL, but while there was no difference in VCA titers between the BL cases and the many simultaneously collected controls, antibody to the early antigen appeared to be important in predicting who would develop BL. The study is continuing with a larger number of cases and controls under analysis. Evidence for an infectious component to the etiology of BL was provided by additional clustering of BL cases and the finding that BL has a seasonal onset. The likelihood that Burkitt's lymphoma has more than one etiology was provided by a contract with Litton Bionetics where biopsies from American Burkitt patients were studied in collaborative fashion for the presence of EBV genome. One of five tumors was found to have EBV genome and a cell-line producing EBV was developed from the genome-positive biopsy. This finding is compatible with the report that not all African BL patients have EBV genome in their tumors and therefore EBV genome positive and negative patients occur in Africa and the United States, although the percentage in both continents differs. Seroepidemiological studies performed by the IARC showed striking ethnic population differences in immune response to EBV. Normal Cantonese Chinese males, at high risk of developing NPC, had high antibody titers to the EBV-associated soluble antigen (CF), whereas Ugandans had low VCA titers. A significant number of Ugandans (approximately 9%) reconverted to undetectable antibody levels while decline in antibody was unusual in normal individuals from France and the Far East. Longitudinal studies in NPC and BL patients continued to show a relationship between antibody titer and prognosis, with elevated antibodies to the EBV-associated early antigen (EA) in short-term survivors with NPC and BL. The finding of the EBV genome in the epithelial

cells of NPC biopsies in two separate laboratories further strengthened the link between EBV and NPC. A genetic contribution to the etiology of NPC was provided by IARC-supported studies in Singapore, where NPC patients and their relatives were shown to have an HL-A antigen on their leukocytes which were rarely detected in non-Chinese or in Chinese controls. Sero-epidemiologic studies supported by the Aichi Cancer Center Research Institute in Tokyo demonstrated that survivors of the atomic bomb in Hiroshima, Japan, did not have higher EBV titers than controls, thus indicating the unlikelihood that radiation triggers any particular change in the mechanism controlling EBV infection. Immunity to EBV utilizing skin tests as well as in vitro measurements of cell-mediated immunity was pursued by investigators working on the IARC subcontract in Hong Kong, at George Washington University, and at Litton Bionetics. Evidence for specific NPC-associated antigens in HKLY-28 was provided by the positive skin test response of 60% of NPC patients against membrane extracts prepared from HKLY-28-derived antigens, while individuals with other forms of cancer were rarely positive. Lymphoma patients reacted with high frequency to antigens derived from the Raji cell line, which originated from an African Burkitt's tumor. Similar antigens derived from cell lines obtained from normal individuals were not immunogenic in cancer patients with NPC, lymphoma, solid tumors or leukemia.

Long-term epidemiologic studies aimed at detecting clues toward a viral etiology of lymphoma were continued by the National Center for Disease Control (NCDC), Hebrew University, and the International Union Against Cancer (UICC). Studies at the NCDC are aimed at determining whether infectious mononucleosis is a premalignant disease by identifying approximately 7,000 cases of infectious mononucleosis with matched controls at selected U. S. colleges. The studies at Hebrew University are providing data on a large well-defined population with comparable measurements of virus exposure, immunologic status, and genetic background. Analysis of the incidence of the different subtypes of lymphomas in native born Israelis and migrants indicated that there are factors at risk for Hodgkin's disease within the country since native-born Israelis had a higher mortality from Hodgkin's disease than foreign-born Israelis. Another population of great interest for comparison with the Israeli group are the Colombians studied by the UICC. Hodgkin's disease occurs in a more fulminant form and at a younger age in Colombia, which is a developing country, than it does in Israel. By comparing the epidemiology, the immunity to candidate oncogenic viruses, and genetic features (as exemplified by HL-A typing) of the population in Colombia and Israel, common features indicative of a possible viral etiology of lymphoma may be identified.

An animal model for induction of lymphoma by a naturally-occurring horizontally transmitted herpesvirus was pursued at the Delta Regional Primate Center where effective immunosuppression of squirrel monkeys has permitted an opportunity to determine whether Herpesvirus saimiri (HVS) can be oncogenic in the natural host under certain conditions. Careful rearing of newborn monkeys has permitted the isolation of virus-negative animals. Transplacental transmission of the virus was found to be uncommon.

Cellular immunity studies to another herpesvirus, herpes simplex type 2, which is suspected as an etiologic agent for cervical cancer, was initiated at Johns Hopkins University and Emory University. These laboratories have developed lymphocyte stimulation assays, cytotoxicity assays, and lymphocyte-dependent antibody assays which are being integrated into longitudinal studies on cervical cancer patients and controls.

Studies in human breast cancer focused on the cross-reactivity between mouse mammary tumor virus and human breast cancer cells. Three contractors working on this effort, New York Medical College, George Washington University, and Litton Bionetics, independently showed that human leukocytes that were reactive against human breast cancer cells were also reactive in migration inhibition assays against mouse mammary tumor virus but not against C-type RNA viruses. At New York Medical College it was also shown that in situ human tumors were more immunogenic than invasive tumors. Longitudinal studies on immunity in mice with breast cancer were analyzed as a model for human breast cancer by investigators at the University of Miami and at M.D. Anderson Hospital. Details of the immune response to tumor and virus-associated antigens were ascertained in cell-mediated and humoral immunity assays.

Improved methods of controlling leukemia in mice as a model for humans was developed at Mt. Sinai Hospital where combination therapy utilizing drugs, neuraminidase-treated tumor cells, and antiviral agents were successful in the Gross virus-AKR system. Similar protocols were applied to patients with acute myelocytic leukemia, with encouraging results. Decreasing viremia was noted in the mouse system in parallel with more effective chemoimmunotherapy, but collaborative studies at the Scripps Institute indicated that antigen-antibody complexes were found in the kidneys of successfully treated mice more regularly than in animals in relapse. At M.D. Anderson Hospital, formalinized Rauscher leukemia virus was inoculated into cancer patients in an initial attempt to evaluate a possible viral vaccine. A collaborative study between these investigators and scientists at Litton Bionetics indicated that humans, mice, and monkeys develop humoral antibody to formalinized RLV, but only humans were able to demonstrate a cellular immune reactivity. Evidence toward a viral etiology of human leukemia was provided by studies at Johns Hopkins University where acute myelocytic leukemia (AML) cells were found to have antigens stimulating the lymphocytes of family members of leukemia patients. Reverse transcriptase was found in the AML cells by collaborating investigators at the National Cancer Institute.

More general studies on immunity and susceptibility to cancer were carried out by four I-E Segment contracts. At Biotech Labs, skin fibroblast transformation studies confirmed previous reports that SV40 transformation was increased in certain relatives of cancer patients, but it was also shown that an RNA virus, simian sarcoma virus, generally gave less transformation in individuals within cancer families. Cell-mediated immunity studies at UCLA, University of Texas and Litton Bionetics indicated that lymphocytes of most individuals are likely to interact with target tumor cells in tissue culture, thus making achievement of tumor specificity unlikely.

Other activities of the Segment: The Segment participated heavily in the development of a joint Virus Cancer Program-Fogarty International Center symposium on "Immunologic Control of Virus-Associated Tumors in Man: Prospects and Problems." Experienced investigators reviewed the possibilities and problems of developing preventive as well as therapeutic vaccines against human leukemia, lymphoma, cervical cancer and breast cancer.

Dr. Paul Levine, VLLB, DCCP, Chairman
Dr. Gary Pearson, VBB, DCCP, Vice-Chairman
Dr. Clarice Gaylord, VLLB, DCCP, Executive Secretary

AICHI CANCER CENTER RESEARCH INSTITUTE (NO1-CP3-3290)

Title: Immunologic and Epidemiologic Studies on Cancer Patients in Japan

Contractor's Project Director: Dr. Yohei Ito

Project Officer (NCI): Dr. Tadao Aoki

Objectives: (1) To investigate the role of herpes and Type C RNA viruses as possible etiological agents for certain human neoplastic diseases; (2) to supply cancerous specimens from Orientals.

Major Findings: In collaboration with the laboratories at the Atomic Bomb Casualty Commission, in Hiroshima, Japan, studies were initiated to evaluate sera from cancer patients and normal individuals exposed to the atomic bomb. The major objective was to determine whether the combined effects of irradiation and a possible pre-existing virus could be etiologically related to certain human neoplastic diseases. EBV (VCA) antibody titers were measured and the results were that no difference in the virus titers could be demonstrated in either group.

Immunological studies on Rauscher leukemia virus (RLV) were carried out in an effort to establish an experimental model system which could be applied to human systems. By means of indirect immunofluorescence, the presence of cross-reacting antibodies against embryonic cells and RLV was demonstrated in sera from C57bl/6 mice which were previously immunized with RLV containing cells. This finding may suggest that mouse fetal antigens share antigenic determinants with Rauscher leukemia virus. These antigens appeared in the 12-day-old embryo and were maintained until birth. The antigens were also localized and found to appear in the cells of the digestive tract but were not present in cells from the liver and brain. The antibody cross-reactivity with RLV and embryonic cells could be clearly differentiated from IgM autoantibodies in mouse alloantisera.

Studies on RLV reverse transcriptase confirmed that, by analysis of the 150 fold purified preparation, the enzyme exists as an aggregate protein with a molecular weight of 480,000 daltons. This enzyme is converted to 135,000 and 70,000 dalton molecules when exposed to 0.5 MKCl. The removal of the salt by gel filtration permits reversibility to the larger molecular size.

Tissue of nasopharyngeal carcinoma cases in Japan were cultured *in vitro* and inoculated into "nude mice."

Significance to Biomedical Research and the Program of the Institute: This project represents a prime source of tissue and sera from a non-Caucasian population in the Far East. These materials, along with the contractor's studies on immunology and epidemiology, are of considerable value in obtaining data which are complementary to observations made on Caucasian populations concerning antibody distribution to viral antigens and the possible involvement of viruses in human cancer. The relationship of genetics to the viral etiology of cancer is extremely important and can best be worked out in different racial groups at different risks for specific tumors.

Proposed Course: The completion of the studies originally developed by the contractor are resulting in a phase out of this contract on April 30, 1975. Further comparative studies on the immunology and epidemiology of certain tumors in Asia and the United States will be carried out through the existing contract with IARC and new studies will be proposed through the Japan-Hawaii program.

Date Contract Initiated: May 1, 1973

Current Annual Level: $40,000

UNIVERSITY OF ALABAMA (NO1-CP4-3394)

Title: Immunologic Studies on the Relationship of Embryonic Antigen to Virus-Induced Tumor Antigens

Contractor's Project Director: Dr. Eddie W. Lamon

Project Officer (NCI): Dr. Gary Pearson

Objectives: The objective of this contract is to employ *in vivo* transplantation methods and *in vitro* cellular and humoral immunological assays to determine the relationship between embryonic antigens and virus-induced transplantation antigens in animal systems.

Major Findings: (1) In order to determine the magnitude of the cellular immune response to embryonic versus virus-induced tumor antigens, C57bl x DBA F_1 hybrids were immunized against SVA (an H-2^d SV-40 transformed line), Py 3T3 (an H-2^d polyoma transformed line) and primary H-2^d embryo fibroblast culture cells which were pre-tested with mitomycin C. Lymphocytes are being tested after primary, secondary, tertiary, and quarternary immunization with 10^5 embryonic or virus-induced cells to determine the optimum number of immunizations required with each antigen to induce an immune response and to determine quantitative lytic activity. (2) Methods for evaluating mechanisms of tumor cell destruction *in vitro* in the MSV system are being developed, the results of which will have a direct bearing on the evaluation of the cellular immune response to embryonic versus viral specific antigens. IgM as well as IgG from MSV regressor animals was able to induce cytotoxicity by normal lymphocytes and potentiate the activity of immune lymphocytes against target cells bearing the appropriate viral

antigen(s). A heterogeneous population of effector cells seemed to be involved in this tumor-specific antibody-dependent lymphocyte cytotoxicity including normal thymus cells when IgM was used as the sensitizing antibody.

Significance to Biomedical Research and the Program of the Institute: Determination of the association between virus transformation and the expression of fetal antigens and the ensuing host immune response to these antigens will provide important information on the mechanism of viral oncogenesis and also on the feasibility of using fetal antigens in immunological control experiments against virus-induced tumors.

Proposed Course: Investigations will be directed at determining whether the same or different lymphocytes are reactive against embryonic versus viral specific antigens, the kinds of cells (T, B or macrophage), the mechanisms of *in vitro* anti-target cell activity by the lymphocytes, and the *in vivo* significance of each component of the response.

Date Contract Initiated: June 28, 1974

Current Annual Level: $55,646

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP4-3365)

Title: Transformation of Fibroblastic Cells derived from Patients with Genetic Diseases

Contractor's Project Director: Dr. Robert Ting

Project Officer (NCI): Dr. Adi F. Gazdar

Objectives: The major objective of this contract is to explore human genetic variation in susceptibility to transformation by oncogenic RNA and DNA viruses and help identify high cancer risk groups.

Major Findings: This contract has been funded for only a relatively short time, during which the major effort has been devoted to standardizing methodology and exploring variations in transformation sensitivity of normal cells. Whole human embryo (WHE) cells from 3rd-9th passages have been used as standard control cells. Of three RNA transforming viruses, simian sarcoma virus(SSV) was found to be the most efficient for transformation of WHE, and the foci so formed were relatively easy to read. Using the direct focus assay, results of individual tests varied 4-5 fold. An infectious center assay was developed in an effort to minimize this variability. Mitomycin C treated SSV infected cells were plated onto sensitive NRK or WHE cells. This indirect method reduces the variability to twofold. The infectious center method has the additional advantage of giving positive results with slow growing fibroblast lines which are negative by the direct transformation method. Approximately 40 fibroblast lines (mainly controls) have been tested. The efficiency of transformation of these lines varies from 1-50% of that for WHE. SV40 transformation

studies have only recently began. However, WHE cells are relatively resistant to SV40 transformation, but highly sensitive to SSV transformation.

Significance to Biomedical Research and the Program of the Institute: One of the objectives of the Cancer Plan is to identify groups of individuals that are more susceptible to cancer development than the general population. This contract will attempt to identify such groups and develop methods to measure the degree of susceptibility. Thus this contract is of direct importance and relevance to the objectives of the NCI.

Proposed Course: It is proposed that approximately fifty lines each of controls, leukemic cases and their relatives, and various genetic defects be tested for SSV transformation susceptibility. Because of certain technical difficulties, smaller numbers will be tested for SV40 transformation susceptibility. In addition, some 10-15 fibroblast cultures will be established from systemic lupus erythematosus cases, an immune complex disease associated with a greater than normal incidence of lymphoproliferative disorders. These studies should permit an evaluation as to whether groups of people more susceptible to transformation exist and determine whether further studies along these lines are justified.

Date Contract Initiated: June 28, 1974

Current Annual Level: $35,208

UNIVERSITY OF CALIFORNIA (NO1-CP4-3211)

Title: Studies of Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer

Contractor's Project Director: Dr. Paul I. Terasaki

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To detect cellular and humoral immunity in cancer patients and to determine the specificity of these reactions; (2) to understand the cellular components of the immune response to cancer and its interaction with antibodies in terms of resistance or susceptibility to cancer; (3) to study the immune response of cancer patients and normals to different viruses and viral-induced antigens; and (4) to examine the relationship of genetic markers (HLA and others) for linkage to the incidence of cancer in families.

Major Findings: Previous studies concerning the specificity of cell-mediated cytotoxicity against cultured target tumor cells had indicated that specific immune reactions could not be detected because of the reactivity exhibited by controls including healthy persons and patients with other types of cancers. This discrepancy led to the study of the mechanisms of cell-mediated cytotoxicity against cultured tumor cells in man. In examining the causes of this nonspecific cytotoxicity for targets, fractions of the effector suspensions were studied. Heparin was added to all tests because previous

reports have shown that granulocytes or their sonicate cause target cells to detach nonspecifically and that this effect is inhibited by heparin. Comparative studies were performed with the microtest plate I (Takasugi), the microtest plate II (Hellstrom), and a semi-micro adaptation of the ^{51}Cr-release assay. The same effector suspensions were tested on target 696 (2T) and good correlation was observed among all three tests. The conclusion drawn is that the same basic phenomenon is detected by the three tests.

Effector cell suspensions were fractionated on degalan columns. Loss of reactivity was observed for all eluate cells regardless of the treatment of the column. Cytotoxicity was weakest with a higher percentage of E-rosetting cells obtained from the coated columns, suggesting that B cells are cytotoxic. Loss of reactivity with uncoated columns indicates either the presence of monocytes in the original suspension or the retention of B cells in the column.

The study of cell types was performed with effector cells from normal persons and illustrated the contribution of different cell types to the background effect. Since granulocyte effects were excluded by the addition of heparin, monocytes and B cells probably accounted for most of the target cell reduction. Specific immune reactions, if they exist, might be detected in the T cell fraction.

A microassay system utilizing lysis of infected cells has been developed for studies of immunity to herpes hominis types I and II.

Previous indications of associations of cancer incidence in families with HL-A haplotypes suggested that immune responsiveness linked to HL-A haplotypes might explain the relationship. The cell-mediated cytotoxic response (MRT) was studied among siblings in healthy families for a possible linkage to HL-A haplotypes. Results showed that the differences in MRT reactivity among siblings did not correlate with haplotypes.

HL-A association studies have shown some differences between various types of cancers. Although the difference with control populations has not been great, there appear to be definite HL-A links with different types of cancers. More detailed haplotype analysis is being performed on all cancer patients.

Significance to Biomedical Research and the Program of the Institute: This contract attempts to relate humoral and cellular immunity against tumor and viral-associated antigens to susceptibility to cancer and prognosis of disease. The ability of normal and cancer patients to respond to tumor and/or viral antigens is being studied for linkage to histocompatibility and other genetic markers. Thus, the successful demonstration of specific immunity in cancer patients will have therapeutic implications and the immunity profiles of normal family members and controls may provide clues to the differential susceptibility of individuals to cancer.

Proposed Course: (1) Studies on the specific T cell cytotoxicity and the involvement of different cell types in nonspecific cytotoxicity will be further examined; (2) studies on the immune specificity among persons with recurrent herpes II, no detectable herpes antibody, and cervical cancer will be carried out utilizing the newly developed microassay system for HS I and II; (3) HL-A associations will be continued in a larger series of patients, including Hodgkin's patients from Israel and Columbia.

Date Contract Initiated: July 12, 1971

Current Annual Level: $298,275

CENTER FOR DISEASE CONTROL (NO1-CP4-0202)

Title: Epidemiologic Studies of Leukemia and Related Diseases

Contractor's Project Director: Dr. Clark Wright Heath, Jr.

Project Officer (NCI): Mr. Roger R. Connelly

Objectives: The objectives of this contract are to relate epidemiologic studies of leukemia and related diseases to laboratory assays pertinent to the viral etiology of cancer: (1) ad hoc field investigations of community clusters of cancer cases, unusual viral illnesses, and/or animal tumor-associated cases; (2) multiple-case family studies; (3) cohort study of heterophile-positive infectious mononucleosis cases with respect to subsequent cancer risk; and (4) epidemiologic study of interpersonal contact in relationship to leukemia and lymphoma incidence.

Major Findings: Among a number of ad hoc investigations undertaken were the following: (1) a reported cluster of human leukemia cases associated with a lymphomatous dairy herd in rural New York State could not be confirmed. However, cases of bovine lymphoma were confirmed in some of the cattle, and high antibody titers to a bovine "C" particle were found in 1 such cow at autopsy. Only 1 human case of leukemia could be confirmed--a man living on the farm whose wife died of breast cancer; (2) acute lymphocytic leukemia occurred in 6 children in 3 counties in southeastern South Dakota where the expected incidence is 0.5 cases. No unusual associations were found linking these cases together beyond their apparent time-space clustering. No environmental hazard of increased viral illnesses were found; (3) four persons at Sloan-Kettering Institute, 2 working with human cell cultures from patients with warts and 2 working with cells cultured from Hodgkin's disease patients, complained of a clinical syndrome of fever, malaise, and lymphadenopathy. Two other persons working with the Hodgkin's material developed diarrhea and a third developed herpes zoster. Another laboratory worker without known direct contact with the Hodgkin's material also developed zoster. Serologic studies on sera of ill and well persons, as well as those done on sera from the Hodgkin's disease patients, revealed no consistently increased titers to various known herpes group viruses; (4) four new cases of leukemia or lymphoma were diagnosed in residents of Fort Dodge, Iowa. All ill persons were interviewed and could be connected by weak or

direct interpersonal contact. However, no contacts could be demonstrated to exist between this cluster and an earlier group of cases (1967-1973); (5) of 5 females working in a laboratory with an algal virus, LPP-ID, 2 have developed carcinoma of the breast and 2 benign nodules on their extremities. This situation is currently being evaluated through review of medical records.

Multiple-case family studies continued to focus on the use of HL-A typing and virus antibody studies in tests for susceptibility to cancer. Two of the families entered into the study this year had more than four first-degree relatives with cancer.

A determination of whether heterophile-positive infectious mononucleosis is followed by a significantly increased incidence of cancer continued with the collection of records from 1400 cases and 1800 controls from the University of California at Berkeley.

Evaluation of interpersonal contact as a possible risk factor for leukemia and lymphoma in Connecticut neared completion. Twenty-six teachers were identified as having either a leukemia or lymphoma diagnosed during the study period 1960-1971. All teachers have been traced through state office records to document dates and places of high school teaching in Connecticut. Follow-up of the students, however, is still in progress.

Significance to Biomedical Research and the Program of the Institute: These various epidemiologic studies are designed to provide clues regarding the potential importance of infectious agents for the etiology of cancer. This is being done by seeking patterns within multiple-case families and community clusters, by defining features of possible premalignant states (IM), and by assessing the potential importance of interpersonal contact for specific kinds of leukemia and lymphoma. Should objective epidemiologic evidence suggesting an etiologic role for infectious agents emerge in any of these settings, specific intensive longitudinal laboratory/epidemiologic studies would be indicated. Specimens collected in the course of these epidemiologic studies provide highly characterized material of particular importance to laboratory workers in the Virus Cancer Program.

Proposed Course: It is expected that the contract will continue as in the past with ad hoc field investigations, intensifying efforts for combined laboratory-field studies, particularly with respect to multiple-case families. It is planned to complete the analysis of interpersonal contact clustering in Connecticut and the infectious mononucleosis follow-up study by the end of fiscal year 1975.

Date Contract Initiated: July 1, 1967

Current Annual Level: $120,576

CHILDREN'S HOSPITAL OF PHILADELPHIA (NO1-CP3-3272)

Title: The Propagation and Seroepidemiology of EB Virus

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The objective of this contract is to determine the relationship between EBV and human cancer. Studies on this contract include: (1) improvement of existing and development of new techniques for the detection of EBV related antigens and titration of corresponding antibodies; (2) searching for methods to measure cell-mediated immune reactions in EBV-associated diseases and, if successful, their application to detection of blocking (tumor enhancing) factors; (3) determination of frequencies and titers of antibodies to various EBV-determined antigens in EBV-associated diseases, with emphasis on the longitudinal study of patients in efforts to detect disease-related differences in antibody patterns, and to ascertain whether changes in the spectra and titers of various antibodies are referable to preceding or subsequent clinical events, thereby providing prognostic information and support for a casual relation of EBV to given human malignancies; and (4) attempts to improve yields of infectious virus from present sources and search for new sources in efforts to obtain potent EBV preparations originating from various EBV-associated diseases or healthy carriers, for comparison of their antigenic and other biologic properties.

Major Findings: Studies in the past year concentrated on the relationship of EBV-associated nuclear antigen (EBNA) to various EBV-associated diseases. A new procedure was developed because of the finding of false negatives with anticomplementary sera. Antigenic preparation was improved by fast drying and minimal exposure to PBS during the staining procedure.

In addition to technical improvements in the assay, studies showed: (a) EBNA is demonstrable in the carcinoma cells of NPC biopsies and not in the lymphoid elements (studies performed in collaboration with Dr. John Ho, IARC); (b) EBNA-free lymphoblastoid cell lines from histiocytic lymphomas have been identified (cell lines supplied by Dr. Henry S. Kaplan); (c) continual search for EBNA positive cells in the peripheral blood of infectious mononucleosis patients failed to reveal EBV antigen. The use of EBNA antibody may be implemented for the detection of infectious mononucleosis since EBNA appears several weeks after VCA, MA, EA and neutralizing antibody; (d) EBNA may serve as a close correlate of cell-mediated immunity to EBV-associated antigens. In addition to EBNA antibody first appearing at a time of apparent active CMI responses to EBV-associated antigens and infectious mononucleosis, low titers of EBNA were found in Hodgkin's disease patients who are known to have poor cell-mediated immunity and high titers to other EBV-associated antigens. Renal transplantation patients also have poor cell-mediated immunity and were noted to have particularly low anti-EBNA titers.

Evaluation of other EBV-associated assays revealed the following: (1) lymphoblast extracts were shown to have two components in the immunodiffusion tests which were unrelated to EBV and which must be considered in efforts to separate and purify EBV-related antigens; (2) a heat labile complement dependent cytotoxic antibody for lymphoblast was shown to be unrelated to EBV. Neutralizing antibody continues to correlate with antibodies to the membrane-associated antigen whether the neutralizing antibody is based on EA synthesis, colony formation or transformation of exposed cells (study performed in collaboration with Dr. George Klein and Dr. John Pope).

Longitudinal studies in Burkitt's lymphoma, which have previously concentrated on BL patients in Nairobi, have now been pursued in Kampala (Drs. Olweny and Magrath) and Ghana (Drs. Nkrumah and Depue). Anti-EA titers (mainly anti-R) appear to have a poor prognostic implication. A wide range of anti-EBNA titers have been found and interpretation is not yet quite clear.

Cooperation in the prospective study in the West Nile district of Uganda indicates that patients have EBV antibodies prior to the onset of BL when serum is collected between five and eleven months prior to diagnosis. This blind test, which included numerous control sera, raises the possibility that high anti-EA titers are of greater predictive value for BL than the VCA assay. These findings correlate with a study in collaboration with Dr. Ian Magrath; a patient developed Burkitt's lymphoma nine months after diagnosis and successful chemotherapy of Hodgkin's disease. Anti-VCA has been present throughout the course of the Hodgkin's disease but a high persistent anti-EA titer and a moderate anti-EBNA titer coincided with appearance of Burkitt's lymphoma.

In nasopharyngeal cancer, anti-EA also appears to parallel prognosis in continuing longitudinal studies performed in collaboration with Dr. John Ho. Declines in anti-D have become apparent in long-term survivors whereas several deaths have occurred among those who developed and maintained high anti-D titers. The great majority of these patients also have anti-EBNA titers (greater than or equal to 1:40). In the NPC studies, antibody patterns in American and Swedish NPC patients appears to be similar to Chinese NPC as determined in the collaborative studies with Dr. Brian Henderson and Andre de Schryver.

Significance to Biomedical Research and the Program of the Institute: The primary purpose is to prove the etiologic relation of EBV to certain human malignancies. Fingerprints of EBV have been found in nearly all BL and NPC biopsies. This virus transforms normal lymphoid cells in vitro into permanently growing lymphoblasts which have malignant properties and are indistinguishable from cultured BL cells. EBV may induce lymphoproliferative malignancies in several non-human primate species. Patients with BL or NPC generally have antibodies to EBV-related antigens, often at very high titers, and changes in the spectra and titers of various antibodies are referable to clinical events. EBV-related serology thus may serve to detect advancing disease, to provide prognostic information, and to monitor the

effectiveness of therapy. Despite the mounting evidence for a causal relation of EBV to BL and NPC, present data do not rigidly exclude a passenger role of the virus in these malignancies. Furthermore, if the role of EBV were causal rather than casual, other factors must undoubtedly be operative to permit this common virus to express its evident oncogenic potential. Among these factors immunologic defects deserve special consideration. The types of antibodies studied most extensively are directed against EBV structural and EBV-induced intracellular antigens which could not affect the tumor. It is most essential, therefore, to search for humoral and cell-mediated immune responses directed against EBV-transformed cells. If successful, methods would become available for detection of blocking (or tumor enhancing) factors. Pertinent studies on IM patients may reveal how they control EBV-transformed cells. It then can be determined whether this mechanism fails to materialize on rare occasions or becomes inoperative under certain circumstances so that transformed cells escape control. Such evidence would significantly strengthen a causal role of EBV in human malignancies, and provide a firmer basis for consideration of EBV-specific preventive measures.

Proposed Course: (1) Continued emphasis will be placed on the improvement of existing assays (particularly EBNA) and the development of new assays for the measurement of humoral and cell-mediated immunity to EBV-associated antigens.

(2) Longitudinal studies on BL patients in Nairobi, Uganda and Ghana will be continued to provide further evidence for the prognostic significance of EBV-related antibody patterns and to determine especially the relationship of anti-EBNA titers to clinical events. Normal controls will be studied in parallel. Cell-mediated immunity studies will be performed on Uganda and Ghana patients which will be compared with the serologic patterns.

(3) The NPC studies in collaboration with Dr. John Ho in Hong Kong will require at least two more years until most of the patients have passed the five year mark. Also in collaboration with Dr. Ho, the specificity of the EBNA antigen in NPC biopsies will be pursued by evaluating biopsies from patients with other carcinomas and tumors of the head and neck as well as normal tissues adjacent to the tumors. Other studies will include the relationship of EBV titers and T cell function in studies on renal transplantations.

Date Contract Initiated: March 1, 1973
(This is a continuation of contract PH 43-66-477 initiated February 2, 1966.)

Current Annual Level: $161,080

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP4-3395)

Title: Immunological Studies on the Relationship of Embryonic Antigens and Virus Associated Antigens

Contractor's Project Director: Dr. Samuel A. Wells

Project Officer (NCI): Dr. Gary Pearson

Objectives: The major objective of this contract is to evaluate the immunological similarity known to exist between fetal cell antigens and the antigens of tumor cells induced by oncogenic and endogenous viruses.

Major Findings: (1) Eight tumors have been induced in C57bl/6 mice by the Schmidt-Ruppin strain D (SRD) virus. Of these, in vivo growth response curves and immunogenicity of four (SRD-1, SRD-2, SRD-3, SRD-5) have been studied. Electron microscopy of tumor bits has demonstrated both C-type and B-type virus particles to be present.

(2) The tumor lines have been evaluated in vitro for the presence of murine (gp71 and p30) and avian (p27) viral polypeptide antigens by radioimmunoassay. Surface localization of murine gp71 and avian gp85 glycoprotein antigens has been confirmed by using rabbit anti-gp71 and anti-gp85 sera. Further, cytotoxicity assays have been conducted using specific tumor immune sera against each tumor line and normal murine fibroblasts.

(3) Current studies include absorbing tumor specific immune sera with murine and avian virus particles and with second trimester syngeneic fetal tissue to define viral, fetal, and tumor specific antibody titers. Chickens will be injected with the four tumor lines in attempt to recover the SRD genome.

Significance to Biomedical Research and the Program of the Institute: The hypothesis of derepressed host genes being responsible for oncogenesis is being actively studied by several groups in cancer-related programs. The relationship between fetal antigens and virus-associated antigens, particularly those associated with endogenous virus expression, is an important issue in relation to the question of etiology as well as to programs concerned with prevention and control of virus-associated cancers. To better understand the mechanism of neoplastic transformation, it is essential to develop the tools to distinguish between host gene re-expression and new viral gene products.

Proposed Course: Tumor transplantation resistance will be studied in vivo by evaluating tumor growth in (a) C57bl/6 mice immunized appropriately with syngeneic fetal tissues and control tissues, (b) C57bl mice immunized with syngeneic solubilized fetal cell antigens and control tissue antigens and (c) primiparous and multiparous C57bl/6 mice. Further characterization of the relationship between fetal cell antigens and virus-induced antigens will be studied using a variety of in vitro techniques. Monospecific reagents prepared against murine and avian C-type virus components will be used to determine the expression of endogenous virus antigens in mouse cells transformed by Rous sarcoma virus.

Date Contract Initiated: June 28, 1974

Current Annual Funding Level: $77,624

EMORY UNIVERSITY (NO1-CP4-3393)

Title: Cellular Immunity Studies to Herpes Simplex Associated Antigens in Cancer Patients and Controls

Contractor's Project Director: Dr. Andre Nahmias

Project Officers (NCI): Dr. Alan Rabson and Dr. Jose Costa

Objectives: The major objectives of this contract are: (1) to provide the necessary immunological infrastructure required to evaluate any prospective HSV vaccine for human use; (2) to obtain evidence corroborating the hypothesis that HSV-2 plays an etiological role in human cervical neoplasia; and (3) to provide a better understanding of the host factors involved in primary and secondary HSV infection.

Major Findings: Lymphocyte stimulation tests and an assay for interferon, have been successfully adapted to micromethod systems. Purified virion and protein antigens are now being prepared.

A test for leukocyte inhibition factor (LIF) is being developed for HSV antigens. An assay presumed to measure T-cell cytotoxicity against human amnion cells infected with HSV was examined. Results showed that the mononuclear cells had not been washed free of HSV antibodies, so that, in fact, antibody-dependent lymphocyte (ADL) cytotoxicity was being measured. Testing of prostate cells chronically infected with HSV in an effort to determine whether T-cell cytotoxicity can be demonstrated is underway. If successful, this test will be applied to clinical studies.

Further studies in the ADL cytotoxicity system, using HSV infected target cells, have demonstrated that the effector cells are neither T nor B cells, and that the antibody mediator is of the IgG class. Attempts to reproduce this assay, using either of two cervical cancer cell lines, have been unsuccessful. This may be due to excessive (30-45%) background ^{51}Cr-release over the 18 hr. incubation period. Investigations using technetium 99m (^{99m}Tc) as a radioisotopic label are underway as this has been reported to show little spontaneous leakage over 48 hr. incubation.

Results obtained with the complement-dependent antibody cytotoxicity assay have been encouraging since the assay requires only a 2 hr. incubation and ^{51}Cr background leakage is minimal ($<10\%$).

Blood from twenty-seven women with various stages of cervical neoplasia, and eight individuals with recurrent HSV-2 infections has been tested in lymphocyte stimulation assays with PHA, and HSV-1 and HSV-2 antigens. The results are now being correlated with clinicopathological data. Whereas lymphocytes of patients with recurrent HSV-2 infections responded to HSV antigens, lymphocytes from about half of the patients with cervical neoplasia failed to respond, despite the presence of HSV antibodies in the serum.

Significance to Biomedical Research and the Program of the Institute: Most of the evidence supporting an association between HSV-2 virus and cervical carcinoma has come from epidemiological and immunological studies. The immunological studies have concentrated on detecting antibodies in the sera of cancer patients and controls directed against a variety of viral antigens. Comparatively little effort has been directed at examining the cellular immune response of cancer patients and controls to these antigens. The demonstration of a cell-mediated response to HSV-2 in patients with cervical carcinoma can add further support for the etiological link between the herpesvirus and cancer. The use of current or newly developed cellular immunity assays to test the patients' response to a variety of characterized viral antigens, and the correlation of the data so obtained to serological assays and to the clinical status of the patients as well as to the pathology of the lesion will provide understanding of the host factors involved in HSV-2 infection and cervical cancer. This information may be crucial in providing diagnostic and therapeutic aids in patients with cervical carcinoma.

Proposed Course: To continue developing CMI assays specific for HSV-2 as part of the effort to determine the role of HSV-2 in cervical carcinoma.

Date Contract Initiated: June 28, 1974

Current Annual Level: $97,147

GEORGE WASHINGTON UNIVERSITY (NO1-CP2-3251)

Title: *In Vivo* and *In Vitro* Studies of the Immune Response to Virus-Associated Antigens in Lymphoma and Breast Cancer Patients and Controls

Contractor's Project Director: Dr. T. Crandall Alford

Project Officer (NCI): Dr. Ronald B. Herberman

Objectives: (1) To evaluate the cellular immune response of patients with malignant diseases to tumor and virus associated antigens by means of *in vitro* assays and skin tests; and (2) to correlate the results of the skin test and *in vitro* assays with the stage and course of the patient's malignant disease.

Major Findings: Studies on Patients with Breast Cancer. With the direct leukocyte migration inhibition (LMI) assay employing 3M KCl extracts of breast carcinoma tissue and mouse mammary tumor virus (MTV) as antigens, a total of 21/59 bloods from breast carcinoma patients gave migration indices of 0.80 or less, whereas only 3/27 normal individuals gave positive results. Further results employing mouse milk containing MTV demonstrated that 8/19 breast carcinoma patient bloods reacted with either 1:100 or 1:1000 dilutions (equivalent to approximately 10^8 - 10^9 virus particles) of the virus in RIII milk, whereas only 1/20 of the patients' bloods reacted with the MTV-negative milk (RIIIf). No reactivity of these virus positive or

control milk reagents was noted using leukocytes of normal individuals. These findings suggest the possibility of shared specificities between a MTV-associated antigen and a tumor-associated antigen(s) of human breast carcinoma.

Thirteen patients were also skin tested with 3M KCl and hypotonic membrane preparation (HMP) extracts of breast cancer at the time LMI was performed. However, the incidence of positive reactions in LMI was considerably less than skin test reactivity and over-all there appears to be no correlation between the two assays.

The level of T-cells as measured by spontaneous rosette formation with sheep red blood cells serves as a useful indicator of the immune status of cancer patients. The percentage of T cells that form rosettes decreased in most cancer patients and were markedly depressed in patients with disseminated cancer. However, in breast cancer patients (stage I+II) the discrimination was not so clear-cut and T-cell levels were about the same in patients with benign breast tumors.

Studies on Patients with Lymphoma. Cell-mediated immunity (CMI) in one-hundred and five individuals has been assessed and correlated with immunity to Epstein-Barr virus (EBV) and with immunity to membrane antigens isolated from both normal and malignant lymphoid cell lines. Between 10% and 20% of patients with lymphoma, myeloma or carcinoma had depressions of CMI. The most common abnormalities were either a decrease in T cells or impaired responses to phytohemagglutinin. CMI and humoral immunity to EBV-associated antigens were detected in both normal controls and patients with malignant disease. No differences in CMI to EBV were noted among the study groups. Anti-EBV antibody titers were somewhat elevated in all groups with malignant disease. Both normals and patients had a high frequency of CMI to lymphoma membrane antigens. Unlike all other groups, patients with multiple myeloma had cell-mediated immunity to KCl extracted antigens isolated from the normal lymphoid cell lines. Serial assessment of general immune competence and of immunity to tumor-associated antigens are in progress.

Significance to Biomedical Research and the Program of the Institute: Human tumors probably contain tumor-associated antigens. Some data suggest that several malignant diseases, such as lymphoma and breast cancer are caused by oncogenic viruses and one human virus, the Epstein-Barr virus, has been implicated in at least two human tumors. The contractor's studies are designed to assay cellular immunity to virus-associated and tumor-associated antigens, and to correlate immune responsiveness with the clinical stage and course of the patient's malignancy. These data may help to clarify the relationship between immune response to specific viral and tumor antigen, tumor load, and clinical disease. The data may also help clinicians to detect early cancer, to monitor therapy, and to determine relapse or recurrence of malignant disease.

Proposed Course: Longitudinal testing of the immune response of breast cancer and lymphoma patients will be continued and the results of these tests will be correlated with the clinical course and stages of disease. Differences in disease-related response to tumor-associated antigens will be exploited to search for possible oncogenic viruses.

Date Contract Initiated: April 13, 1972

Current Annual Level: $80,000

HEBREW UNIVERSITY-HADASSAH MEDICAL SCHOOL, JERUSALEM (NO1-CP3-3342)

Title: A Multidisciplinary Study of Hodgkin's Disease in Israel

Contractor's Project Director: Dr. Natan Goldblum

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To investigate the interrelationship between environmental and genetic factors in the etiology, pathogenesis and spread of Hodgkin's disease in Israel and, in particular: (1) to evaluate clustering among social contacts, in space and time, and to develop methods for the study of clustering; (2) to evaluate Hodgkin's disease in immigrant and native-born Israelis, including studies of HL-A typing, and EBV and other virus antibodies; (3) to review the histological sections of all malignant lymphomas diagnosed throughout Israel during 1964-1972 so as to provide basic information necessary for the immunoepidemiologic studies; and (4) to establish laboratory assays relevant to an understanding of the pathogenesis of malignant lymphoma.

Major Findings: An analysis of mortality among the Jewish population of Israel in 1950-71 showed that the death rate from non-Hodgkin's lymphomas rose during this period. This rise occurred among older adults in all population groups and among children and young adults born in Africa. Adult mortality from Hodgkin's disease rose less markedly, and not among persons born in Europe, whose mortality decreased in this period. The increase in mortality from both Hodgkin's disease and other lymphomas was higher among native-born Israelis than in the foreign-born groups. The rates among the foreign-born varied with the continent of birth, but no clear-cut relationships were found with age at immigration or during the period since immigration.

Review of histological material from 1960-72 has now been virtually completed. Attempts to trace outstanding cases, especially from 1960-63, are continuing. Incidence rate of histologically confirmed Hodgkin's disease for 1964-68 have been calculated. In young adults, rates are higher in Jews born in Europe-America than those born in North Africa or Asia. The Rye-type frequencies in 327 cases of Hodgkin's disease in adults are as follows: nodular sclerosis, 45%; mixed cellularity, 28%; lymphocytic depletion, 17%; lymphocytic predominance, 10%.

From 90 biopsies, 24 lymphoblastoid cell lines were established. The majority were derived from lymph-node biopsies and spleens of patients with Hodgkin's disease; some came from patients with other malignant lymphoma. All lymphoblastoid cell lines were found to be B cells; of twelve cell lines tested for the presence of EB nuclear antigen (EBNA), all but one were positive. The EBNA negative cell line is under further study. All the established lymphoblast cell lines have a significantly reduced ability to form polar caps with fluorescent Concanavalin-A. One of the cell lines, isolated from a lymph-node biopsy of a patient with Hodgkin's disease, seems to be morphologically different from all the other lymphoblastoid cell lines.

In biopsy material from Hodgkin's disease patients, a significant decrease in the ability of the lymphocytes to form caps was observed. Lymphocytes isolated from tonsils of "control" patients undergoing tonsillectomy and from axillary lymph nodes of breast cancer patients exhibited approximately 30% of cells with caps, which is identical with the cap-forming ability of normal lymphocytes. This difference in the mobility of Con-A sites was even more pronounced in lymphocytes isolated from the peripheral blood of lymphoma patients, as compared to lymphocytes from healthy donors. In 122 patients with Hodgkin's disease and other malignant lymphomas, cap formation ranged between 4 and 10%. These findings may be of importance as an aid in the differential diagnosis of Hodgkin's disease and other malignant lymphoma.

An EBV (EBNA) negative cell line has been established from the pleural fluid of a child with intestinal lymphoma. The cells, unlike lymphoblastoid cells grown in culture, have a shorter generation time and grow to very high cell densities. In addition to being EBNA negative, the cells apparently lack B and T receptors and have IgM-kappa. Additional childhood intestinal lymphoma cases are being collected and studied.

Significance to Biomedical Research and the Program of the Institute: A comprehensive study of all lymphoma cases arising in a large, well-defined population, with comparable measurements of virus exposure, immunologic status, and genetic background on a control group, is needed by the Program to determine the relationship of environmental and genetic factors in the etiology of this disease. The opportunity afforded by this well-identified population offers an excellent possibility of determining the cause of Hodgkin's disease and other lymphomas. The mortality findings are consistent with the hypothesis that environmental, probably infective, factors play a role in the etiology of both Hodgkin's disease and other lymphomas. The reviewed and "Rye-classified" cases of Hodgkin's disease will provide accurate histological information on the cases included in the interview study. They will also enable one to determine the incidence rates and the frequency of the various histological types to be determined for the various population groups in Israel.

Proposed Course: (1) To complete and analyze epidemiologic and histopathologic data collected on Hodgkin's disease patients diagnosed between 1960 and 1972 and on their matched controls; (2) to perform HL-A typing, to establish cell cultures from biopsied materials and to determine viral antibody titers on all newly diagnosed cases of lymphoma; and (3) to further investigate the relationship of the "Concanavalin-A probe" with the immune state of Hodgkin's disease patients and as a potential tool for detecting susceptibility to lymphoma in normal individuals.

Date Contract Initiated: June 29, 1973

Current Annual Level: $211,452

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP4-3273)

Title: Sero-epidemiologic and Laboratory Studies on Nasopharyngeal Carcinoma and Burkitt's Lymphoma

Contractor's Project Director: Dr. G. Blaudin de Thé

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The overall objective of the contract is to evaluate the role of the Epstein-Barr virus (EBV) in the etiology of Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). The first specific objective is to carry out a prospective study in a child cohort population of 35,000 in the West Nile District of Uganda. The children were to be bled and then followed for at least five years in order to obtain pre- and post-BL sera. Serological studies on sera of those children developing BL are testing the validity of four hypotheses which have been formulated regarding the etiological relationship between EBV and BL. Two sub-objectives are: (1) to study the role of chronic malaria in the development of BL; and (2) to investigate the epidemiological behavior of EBV infection in the study population by follow-up of randomly selected groups. The second specific objective involves the investigation of the sero-epidemiological behavior of EBV in populations at different risk for EBV-associated diseases, carried out in Hong Kong, Singapore, Uganda, and Tunisia. The third objective is the investigation of the possible role of EBV in the development of NPC through the integration of field and laboratory studies.

Major Findings: (1) Etiological Studies on BL in East Africa: The collection of 35,000 sera from the West Nile population of Uganda was completed in children up through 8 years of age. Six prebled BL cases have been detected and were tested by Drs. Werner and Gertrude Henle of Children's Hospital of Philadelphia. EBV VCA antibodies were detected in pre-disease and control sera, and EA titers appeared to be disease related and of predictive value. The rebleeding of population samples in the West Nile District took place as planned, and included 12 groups of 75 children and their families. There appears to be a seasonal variation in the onset of BL: significantly more cases occur in the second part of the year when the heaviest rain falls. A BL cluster was found along the Oru river, involving 9 of the 20 BL cases

detected in the West Nile District during a 12-month period. This points strongly to some environmental factor as a trigger for BL development. Antibody titers against human herpesviruses, as measured by immunofluorescence in BL cases and controls, indicated that BL patients had elevated antibodies not only to EBV but also to cytomegalovirus (CMV) and varicella-zoster virus (VZV). The response to measles vaccination will be followed by determining measles antibody titers in children who were vaccinated and correlating the response with intensity of malaria infection. The measles antibody testing will be done at the East African Virus Research Institute in Entebbe, Uganda. Malaria parasite counts in the blood is nearly double in boys as opposed to girls between 0 and 9 years of age, the overall average being 2,680 parasites per mm^3 for boys, versus 1,479 for girls. There appears to be a correlation between malaria counts in the blood and viral capsid antigen (VCA) antibodies, those with high VCA titers also having high parasite counts. Retesting of BL cases and controls for sickle trait in the West Nile showed no significant difference between the two in a blind trial. BL registration was continued in the high and low areas of the North Mara region of Tanzania. A BL Working Group Meeting took place in Lyon to assess the feasibility of carrying out a study in Tanzania on the relationship between malaria and BL. Due to many complicating factors it was decided to defer initiating this study.

(2) Sero-epidemiology of EBV: The collection of sera was completed among representative samples of the Chinese, Indian and Malay populations in Singapore, Chinese in Hong Kong, substudy groups in Uganda, and over 2,000 persons in Nancy, France. The age of primary infection by EBV differs in the various groups studied. In the 2 to 3 year age group, 97% of Ugandans and 30% of Singaporean Chinese were VCA positive. After 5 years of age Ugandan males show a serological 'reversion'-i.e., a rising number of VCA negative individuals. The geometric mean titers (GMT) of positive sera for VCA and complement-fixing (CF) antibodies differed in the four groups studied: Ugandans have a very high VCA humoral response to EBV up to the age of ten, and thereafter are lower than any other group studied. Singaporean Indians have the highest VCA response. The prevalence of humoral response against the CF/soluble (S) antigen is higher in Chinese than in other groups studied up to the age of 25 years. The GMT of S antibody is also highest in this group.

(3) Nasopharyngeal Carcinoma (NPC) Program: NPC patients of different ethnic origin (Chinese, Malays, Tunisians) were HL-A typed, as well as some Chinese NPC family members. The previously observed deficit in the second sub-locus HL-A antigen in NPC patients in Singapore was found to segregate in the manner of an HL-A antigen, is not disease induced, and is believed to be directed against a new NPC-related antigen. The possible discovery of a new HL-A antigen in the second locus was achieved using sera from pregnant women in Singapore. The resulting activities could account for the previously reported HL-A antigen deficit. HL-A testing of Tunisian NPC patient lymphocytes showed that the deficit in the second sub-locus HL-A antigen was also present, but to a lesser extent. Immunological studies [cell-mediated immunity (CMI) and skin testing] were developed during the survey period in Hong Kong and Singapore. Hyporesponsiveness to skin

testing with PPD and decreased in vitro lymphocyte stimulation to PHA were observed in NPC patients in Singapore. Skin testing with membrane extracts showed increased reactivity when extracts from the HKLY-28 cell line were used (NPC-derived lymphoblastoid line). The presence of EBV-DNA in cellular sub-populations (epithelial and lymphoid cells) from NPC biopsies indicated that viral DNA was present mostly in epithelial cells. As regards EBV serology, antibodies against EB-nuclear antigen (NA) and CF/S antigen were evolved in parallel. The antibodies against CF/S antigen were found to be linked to the ethnic origin of the patients.

Significance to Biomedical Research and the Program of the Institute: The objectives of the contract should elucidate the role of EBV in two human tumors, Burkitt's lymphoma and nasopharyngeal carcinoma. The prospective BL project in Uganda aims to find direct proof that a human tumor is caused by a virus. The comparative study of the behavior to EBV in populations at varying risk for EBV-associated diseases has shown that only comparisons based on adequate representative samples of these populations are valid. Initial testing on various ethnic groups shows that indeed there are significant differences among populations having different risks of NPC. The results obtained by blood genetic typing, together with the sero-epidemiological results, may provide the means for detecting high risk groups among a normal population for another human tumor, namely NPC.

Proposed Course: The highest priority will be given to the Burkitt's lymphoma case detection activity in Uganda. Considerable effort will be made to ensure proper coverage of the West Nile survey area by the Case Detection Team (CDT) in collaboration with health centers and hospitals. The in-depth study of the populations surveyed in the sub-study will be continued, including the malaria studies, the EBV sero-epidemiological data collection, and the measles study. A determination will be made whether BL is preceded by periods of malnutrition in the West Nile District.

The program on sero-epidemiology of EBV in populations at different risk for EBV-associated diseases will include collection of 2,100 blood samples from 14 selected population groups in the West Nile District of Uganda. Testing of these sera will be done in Lyon. Antibodies to other herpesviruses in BL cases and controls will be sought, in collaboration with investigators in specialized institutes. The remaining sera collected in the Far East and sera in special studies will also be tested.

The nasopharyngeal carcinoma program will be modified. The epidemiological aspects will be reduced mainly to data analysis. The immunogenetic studies will be extended to determine the nature of the deficit in the second locus, and to investigate further the new HL-A antigen associated with NPC risk. The immunogenetic studies in the Far East and Tunisia will be integrated. Cell-mediated immunological studies will be coordinated among Hong Kong, Singapore, Perth (Australia), Tunis, Lyon, and Paris. The in vitro and in vivo CMI studies will be correlated with serological data.

Date Contract Initiated: June 11, 1970

Current Annual Level: $649,555

INTERNATIONAL UNION AGAINST CANCER (NO1-CP4-3292)

Title: International Investigation in the Epidemiology of Lymphoma

Contractor's Project Director: Dr. Pelayo Correa

Project Officer (NCI): Mr. Roger C. Connelly and Dr. Ernest Plata

Objectives: The major objectives of this contract are: (1) To explore the role of infectious agents, such as Epstein-Barr virus, suspected of playing a role in the etiology of Hodgkin's disease. Sera will be collected and tested for antiviral antibodies in collaborating VCP laboratories, and aliquots will be stored for future studies. (2) To ascertain history of past or concomitant diseases which may have an effect on the immune capacity of the host, such as malnutrition and chronic infections. (3) To study genetic markers which may have a bearing on susceptibility to the disease (HL-A-ABO). (4) To study the immune competence of the patients.

Major Findings: This contract was only recently implemented due to an administrative reorganization of the Instituto Nacional de Cancerologia in Colombia. Three centers were selected (Cali, Medellin, and Bogota) for their clinical and diagnostic facilities and because the three centers combined serve close to half of the population (10 million) of that country. The questionnaire instrument to be used in the interview phase of the study was drafted, revised, and pretested during this preparatory phase. The laboratories in Cali were selected to be the focal point for the collection and shipment of specimens, centralization of data and overall direction. This laboratory under the direction of Dr. A. Duenas, also was selected to do the HL-A typing of the specimen donors. Dr. Duenas during this period visited the laboratory of Dr. Terasaki at UCLA to assure the standardization of the methods used in Cali. Collection of data and specimens began in December of 1974.

Significance to Biomedical Research and the Program of the Institute: This project is expected to contribute considerably to our knowledge concerning the etiology and natural history of Hodgkin's disease and other lymphomas. There have been several attempts to study the etiology of Hodgkin's disease in Europe and the United States. In these countries the disease is characterized by a strong host reaction. In countries such as Colombia, where the host reaction is poor, there have been studies only aimed at the determination of the demographic characteristics of the disease. A viral causation is suspected in the etiology of Hodgkin's disease. While a number of investigators using HL-A frequencies suggest that genetic backgroups may be important to disease incidence, it is also important to consider the immune status of the host prior to and during the course of the disease. The postulated etiologic agent may be one of several viruses.

In Colombia, the disease is markedly different from that seen in the United States; types such as nodular sclerosis are practically unknown, the disease frequently affects very young people, and follows a rapidly fatal course. This unique situation provides an unusual opportunity for investigating an etiologic agent. If an infectious agent is involved, it is obviously acting much more effectively and apparently encountering weaker host defenses. In this situation an agent may be much more easily demonstrated. This project envisions, through progressive stages, to study the genetic and immunologic influences suspected, and then proceed to virological studies. Thus, better knowledge of the etiology and pathogenesis will lead to better prevention and treatment of the disease. The entire project fits well within the major objectives of the Virus Cancer Program and those of NCI.

Proposed Course: To enter into the study approximately 150 cases of Hodgkin's disease and related lymphomas, obtaining the appropriate clinical, demographic, environmental, immunological, immunogenetic, and pathological data to permit statistical confirmation of the pathogenesis of the disease in Colombia and to provide indications concerning the types of cases most likely to yield results in projected virological studies.

Date Contract Initiated: June 11, 1974

Current Annual Level: $20,000

JOHNS HOPKINS UNIVERSITY (NO1-CP3-3337)

Title: Immunologic Reactivity Against Tumor and Possible Virus-Associated Antigens in Families of Leukemic Patients and Controls

Contractor's Project Director: Dr. Paul Anderson

Project Officer (NCI): Dr. Paul Levine

Objectives: (1) To measure anti-leukemia cellular immune reactions in patients, family members, and unrelated controls; (2) to test persons who react to human leukemic blasts for reactivity to murine leukemia virus and simian sarcoma virus, and against virus-infected cell lines; (3) to screen normal sera for spontaneously occurring human anti-leukemia antibody; and (4) to expand collaborative efforts in studying sera from responders to leukemia for activity against cell lines infected with simian sarcoma virus and for antibody to viral reverse transcriptase; and in continuing analysis of human leukemic blasts for evidence of virus nucleic acids and enzymes.

Major Findings: A panel of sera from normal healthy humans is under study which shows highly specific, complement-dependent cytotoxic antibody to human leukemic blasts (LB), primarily those from acute lymphoblastic leukemia (ALL). Using a standard microcytotoxicity assay, these sera have been shown to be non-reactive to a variety of non-leukemia control cells. The pattern of anti-leukemia reactivity, and of absorption studies, suggest the existence of two antigenic specificites in ALL cells which are immunogenic in man.

Studies of cell-mediated immune reactions to LB have been carried out using mixed leukocyte cultures, macrophage migration inhibition factor assays, and ^{51}Cr-release lymphocyte cytotoxicity assays. Responses by patients in remission to autologous LB indicate immunogenic leukemia-associated antigens, and that patients are not tolerant to these antigens. Remission duration and time of relapse were not directly correlated with autologous anti-LB responsiveness.

Most family members and unrelated normal controls reacted to leukemic blasts, and reacted to a variety of morphological cell types, suggesting cross-reactivity or common antigenicity of leukemia associated antigens. No difference in response prevalence could be shown between controls known to be exposed to leukemic patients or cells, and those with no known exposure.

Collaborative studies with the Laboratory of Tumor Cell Biology, NCI, have resulted in the demonstration of virus-like RNA, and of reverse transcriptase, in leukemic blasts obtained from several patients.

Significance to Biomedical Research and the Program of the Institute: Clinical, epidemiological and laboratory studies suggest that acute leukemia in man may be a virus-induced disease. Study of immune reactions to tumor-associated antigens, and the cross-reactivity of such reactions, in patients with leukemia, family members, and in exposed and non-exposed unrelated controls, should be useful in evaluating the role of possible oncogenic viruses in human tumors, and in providing assays for monitoring possible antiviral therapy and immunotherapy.

Proposed Course: (1) To continue to measure anti-tumor cellular immunity in a better-defined group of patients, family members, and unrelated controls, using MLC, MIF and LCA assays. Cells with anti-leukemia reactivity to whole leukemic blasts will be tested for reactivity to antigen extracts of leukemic blasts, and for reactions to murine leukemia virus and to crude antigen preparations from a normal human cell line infected with simian sarcoma virus; (2) to continue and expand studies on patients with acute leukemia and their families, screening for the presence of cytotoxic antibody to leukemic blasts; and (3) to extend collaborative studies with the Laboratory of Tumor Biology supplying relapse leukemic blasts and normal remission cells for biochemical analysis in search of viral nucleic acids and enzymes, and to correlate such findings with studies of immune reactions to the same leukemic blasts.

Date Contract Initiated: May 1, 1971

Current Annual Level: $110,850

JOHNS HOPKINS UNIVERSITY (NO1-CP4-3330)

Title: Cellular Immunity Studies to Herpes Simplex-Associated Antigens in Cancer Patients and Controls

Contractor's Project Director: Dr. Laure Aurelian

Project Officers (NCI): Dr. Alan Rabson and Dr. Jose Costa

Objectives: The objectives of this contract are: (1) to establish whether cell-mediated immunity to HSV-2 antigens can be demonstrated in cancer patients; (2) to identify and characterize the antigens responsible for eliciting the cellular immune responses; (3) to correlate CMI responses with serological assays designed to detect humoral antibody to viral antigens; and (4) to relate the results of these studies to the clinical status of patients and controls.

Major Findings: Two in vitro assays of cell-mediated immunity (CMI) to herpesvirus antigens have been established and are now in routine use. These are lymphocyte transformation (LT) and leukocyte migration inhibition (LMI). Establishment of a cytotoxicity assay is now in progress.

Twenty-four subjects, 12 controls and 12 cancer patients (atypia-invasive cancer) were studied since July 1, 1974. All subjects were assayed by LT and 8 subjects were assayed by both LT and LMI. The antigens studied were: (1) HSV-1 and HSV-2 viruses purified from soluble antigens by centrifugation at 20,000 rpm for 2 hours; (2) AG04, the tumor specific antigen made early in infection and AG-H, its control antigen; and (3) CP-1 and CP-4, soluble antigens made from HSV-2 infected HEp-2 cells by calcium phosphate chromatography and shown to be present in exfoliated cervical tumor cells. Furthermore, in view of the close antigenic similarity between HSV-1 and HSV-2, model systems consisting of rabbits immunized with either HSV-1 or HSV-2 or both, were also assayed both by LT and LMI. Neutralization and complement fixation was done on these subjects' sera in order to determine whether they have antibody to HSV-1 and/or HSV-2 and to AG-4.

The results of these studies were:

LT: (a) Considering a stimulation index (SI) of 2 as the cut-off point, most people (15/24) reacted to both HSV-1 and HSV-2. Studies of specificity must be performed before these responses are tabulated as being HSV-1, HSV-2 or both. Accordingly, the rabbit models (infected with either or both viruses in different order) were established and are now under investigation. (b) Only two patients were studied with CP-1 and CP-4 as it took a few months before these antigens were made. Response was seen in one patient to both antigens and appeared to correlate with the response to HSV-2. (c) None of the controls responded to AG-4 or AG-H; response to AG-4 but not to AG-H was observed in 3/12 cancer patients. The three patients showing positive LT with AG-4 were cases of atypia and two of them did not have AG-4 antibody. Patients with recurrent cervical cancer and positive for AG-4 antibody were negative by LT. These results, although preliminary, suggest that a positive prognosis may be associated with lack of antibody to AG-4 but positive LT responses.

LMI: Responses by LMI correlated with those observed by LT for HSV-1 and HSV-2; most subjects (6/8) responded to both viruses again raising the question of specificity. CP-1 and CP-4 antigens inhibited migration in 2/4

patients studied, with CP-4 causing better inhibition. AG-4 inhibited migration in 1/8 subjects, a patient with atypia who did not respond to AG-4 by LT.

Significance to Biomedical Research and the Program of the Institute: Most of the evidence supporting an association between HSV-2 and cervical carcinoma has come from epidemiological and immunological studies. The immunological studies have concentrated on detecting antibodies in the sera of cancer patients and controls directed against a variety of viral antigens. Comparatively little effort has been directed at examining the cellular immune response of cancer patients and controls to these antigens. The demonstration of a cell-mediated response to HSV-2 in patients with cervical carcinoma can add further support for the etiological link between the herpesvirus and cancer. The use of current or newly developed cellular immunity assays to test the patients' response to a variety of characterized viral antigens, and the correlation of the data so obtained to serological assays and to the clinical status of the patients as well as to the pathology of the lesion will provide understanding of the host factors involved in HSV-2 infection and cervical cancer. This information may be crucial in providing diagnostic and therapeutic aids in patients with cervical carcinoma.

Proposed Course: Future studies will include (1) lymphocyte transformation in the presence of autologous human serum; (2) T and B cell separation in order to determine the type of cells involved in LT or LMI; (3) macrophage depletion in order to determine the role of this cell in the above assays; (4) establishment of indirect migration inhibition assay in agarose using guinea pig peritoneal exudate cells and supernatants from LT assays; (5) specificity analyses for both the LT and LMI assays using rabbit models. Specifically animals were inoculated with various sequential combinations of the two viruses. LT and LMI assays were performed kinetically together with serologic analyses before and after hyperimmunization. Cut-off points of specificity are now being determined; and (6) cytotoxicity assays are being established using immune sera and lymphocytes alone or in combination.

Date Contract Initiated: June 28, 1974

Current Annual Level: $133,715

LITTON BIONETICS (NO1-CP4-3333)

Title: Immunological Assays for DNA and RNA Viruses

Contractor's Project Director: Dr. Bruce Maurer

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The major objectives of this contract are, in collaboration with other I-E Segment contracts: (1) to investigate the etiology of human breast cancer with particular attention to the possible presence of a human breast cancer virus immunologically related to mouse mammary tumor virus and/or Mason-Pfizer virus; (2) to utilize assays of cellular immunity developed by the contractor in support of other I-E Segment contracts, and (3) to provide a means of quality control for I-E Segment contractors using similar immunity assays.

Major Findings: The major effort of this contract has been devoted to collaborative studies with other I-E Segment contracts, particularly UCLA, New York Medical College, and the breast cancer portion of the studies at George Washington University. In collaboration with UCLA, the ability of normal human lymphocytes to attack target cells in tissue culture was confirmed and raised questions about the tumor specificity of this assay. Reproducibility of this assay in the hands of independent investigators on opposite sides of the country was confirmed. Confirmation of the report by New York Medical College and the Institute for Medical Research in Camden, New Jersey that an antigen in human breast cancer cross-reacts in an apparently specific fashion with mouse mammary tumor virus was provided by a blind study using the migration inhibition technique. Antigen extracts provided to clinical investigators at George Washington and elsewhere indicated that the hypotonic membrane extracts, although less immunogenic, are more specific than KCl extracts in detecting tumor-associated antigens.

Significance to Biomedical Research and the Program of the Institute: The ability of this laboratory to perform a number of cell-mediated immunity assays allows a means of controlling the quality of cell-mediated immunity assays performed by other I-E Segment contracts. In addition, this contract can perform assays as a service to clinical contractors who do not have the laboratory expertise to perform these assays themselves. Of particular importance are the preliminary findings of a cross-reacting antigen in human breast cancer and the mouse mammary tumor virus. If confirmed in a larger series, this would strengthen the data indicating a viral etiology for human breast cancer. The successful performance of cell-mediated immunity assays to measure the response to these antigens will be of great importance to studies on immunotherapy and will help in monitoring more conventional means of therapy.

Proposed Course: Cell-mediated immunity (CMI) assays (including leukocyte migration inhibition, ^{51}chromium release cytotoxicity, visual microcytotoxicity, lymphocyte stimulation (LS), and measurement of T and B cell rosette) will be utilized to monitor the CMI status of patients having breast carcinoma and lymphoma. The MI and LS assays will utilize various virion antigens, e.g., EBV, MMTV, MPMV, and preparations of tumor cells containing putative tumor antigens. The sera of these patients will also be characterized relative to the presence of antibodies to the aforementioned viruses.

Extracts prepared from virus and tumor cells will be made available to program scientists as well as to other I-E Segment contractors.

A current file will be maintained on American Burkitt's lymphoma patients and their families to include pertinent clinical information and serum antibody titers to EBV related antigens.

Date Contract Initiated: June 3, 1974

Current Annual Level: $217,000

LITTON BIONETICS (NO1-CP4-3252)

Title: Application of Immunologic Techniques to Studies on the Viral Etiology of Human Cancer

Contractor's Project Director: Dr. William Wallen

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The major objectives of this contract are: (1) to provide virological, immunological and data management support for other I-E Segment contracts, (2) to maintain the American Burkitt's lymphoma registry, and (3) to study the relationship of Epstein-Barr virus (EBV) to human lymphoma.

Major Findings: The major effort of this contract has been devoted to providing supportive services for other I-E Segment contracts, particularly those working in the field of human lymphoma and leukemia. Supportive services rendered include assays for type C virus infectivity, neutralizing antibody, EBV serology, in vitro cell-mediated immunity, propagation of lymphoid cell lines, storage and distribution of samples, and data management. A major effort has been to assay for Gross leukemia virus in the tail veins of mice included in the immunotherapy studies at Mt. Sinai Hospital (Contract # NO1-CP4-3225). These studies show that a decrease in viremia (Gross leukemia virus) correlates with successful chemotherapy, suggesting that it is necessary to control the virus and diminish the chances for viral re-induction in order to obtain effective long term control of leukemia. In collaborative studies with Dr. Evan Hersh (Contract # NO1-CP3-3301), it was found that formalinized Rauscher virus will produce antibodies in rhesus monkeys but not provide an effective cellular immune response. Serologic evaluation of patients at George Washington University (Contract # NO1-CP2-3251) indicated that EBV titers were frequently elevated in multiple myeloma patients and that antibody titers did not correlate with either skin test or in vitro evidence of cell mediated immunity to lymphoma associated antigens.

Thirty-seven new cases of Burkitt's lymphoma were reported to the American Burkitt Lymphoma Registry, bringing the total to 216 cases, 130 of which have been confirmed. The Registry continues to act as a source of clinical material and data for immunological and epidemiological studies concerning the involvement of EBV in American Burkitt's lymphoma.

Significance to Biomedical Research and the Program of the Institute: The major objective of this contract is to provide supportive services for other I-E Segment contracts; therefore, the significance of this work varies from contract to contract. As a general theme, the aim of the contract is to obtain information regarding the role of viruses in human cancer, and to develop methods for the early diagnosis and control of cancer. The relationship of EBV to human lymphoma is not well understood, even though elevated antibody levels have been found in a number of human neoplasms. Studying cases of Burkitt's lymphoma in North America and Africa affords an opportunity to determine whether the African and American diseases are the same, and to compare and identify specific host and environmental factors which might contribute to the disease.

Proposed Course: This contract will be terminated on June 2, 1975 and the research performed on this contract will be divided among other I-E Segment contracts.

Date Contract Initiated: November 1, 1973

Current Annual Level: $251,496

THE MEDICAL COLLEGE OF PENNSYLVANIA (NO1-CP4-3319)

Title: Immunological Characterization of the EBV Antigens

Contractor's Project Director: Dr. Barbara A. Zajac

Project Officer (NCI): Dr. Gary Pearson

Objectives: The overall objectives of this contract are to produce, isolate, purify and characterize various EBV-associated antigens from tissue culture cell lines with the initial emphasis on early antigens (EA), and to produce monspecific antisera against these antigens.

Major Findings: (1) Immunization of two adult rabbits with crude extracts of EBV-infected RPMI 64-10 cells resulted in the production of antibodies to D-early antigens. Antibodies to fetal calf serum and uninfected cell proteins also were detectable (hemagglutination titers of 384 and 2,084, respectively). These extraneous antibodies were removed by use of immunoabsorbents. The antisera were negative when tested for antibodies to VCA, MA and EBNA. One rabbit responded with an anti-D titer of 128 and the other 20. Further immunization with crude extracts did not appear to increase the D-antibody titers. The partial purification of D-early antigens prior to immunization was considered essential.

(2) A blocking immunofluorescence assay was developed in order to follow the purification of the antigens. This assay was adapted for screening for the presence or absence of D-early antigens and made it possible to monitor studies on the properties of D-antigens. D-early antigens containing extracts derived from EBV-infected RPMI 64-10 cells were treated by various procedures and assayed for the presence or absence of EA activity. Sonication of EBV-infected cells resulted in the release into the superna-

tant of the majority of the D-early antigen activity. Some activity remained bound to the particulate fraction and could be solubilized by treatment with KCl. Greater than 90% of D-early antigen activity in crude extracts could be precipitated by dialysis against pH 5.0 buffer or by addition of ammonium sulfate. Dialysis against pH 4.8 buffer caused a partial loss of D-antigen activity. Exposure of the antigen to pH 10.6 resulted in complete loss of activity unless the solutions were brought to neutrality within 10 minutes. D-early antigen activity could withstand dialysis against distilled water and lyophilization. Studies with DEAE-cellulose chromatography revealed that D-early antigen activity could be eluted in two major peaks by using a stepwise increase in salt concentration. These studies have shown that D-early antigens obtained from EBV-infected RPMI 64-10 cells were immunogenic and relatively stable under the conditions tested. The methodology developed provides a basis for the purification of these antigens by a combination of physicochemical and immunological techniques.

(3) In order to improve the amount of D-early antigens available for purification studies, attempts were made to increase selectively the number of cells producing D-early antigens in EBV-infected RPMI 64-10 cells by using IdU or Ara C. The results were not consistently reproducible. Use of 50 to 100 ug per ml of IdU or 0.2 to 2.0 ug per ml of Ara C resulted in some experiments in a two-fold increase in the number of cells producing D-early antigens. In other experiments, no detectable increase or less than a two-fold increase was observed.

Significance to Biomedical Research and to the Program of the Institute: Epstein-Barr virus (EBV) has been increasingly associated with a number of neoplastic diseases including lymphoma and nasopharyngeal carcinoma, primarily on serologic evidence. Specific immunological reagents will be required to investigate the possible etiologic role of EBV in human malignancies. The present work is geared toward preparing, purifying and characterizing viral products detected in EBV-infected cells and toward preparing monospecific reagents directed against these viral products. Such reagents might be potentially useful in the control of EBV infections and, perhaps, EBV-associated tumors.

Proposed Course: Over the next contract year, the research efforts will be directed at the continued purification of D-early antigens and the preparation of a monospecific serum against this antigen. Purification efforts will concentrate on the use of immune affinity chromatography. The homogeneity and physical characteristics of the antigens purified by affinity chromatography will be determined by a variety of methods including immunodiffusion and immuno-electrophoresis, polyacrylamide gel electrophoresis, isoelectric focusing and by molecular weight studies. Experiments will also be directed towards purifying the R component of the EA complex.

Date Contract Initiated: March 1, 1974

Current Annual Level: $86,000

UNIVERSITY OF MIAMI (NO1-CP4-3358)

Title: Immunologic Studies on Animal Breast Carcinoma

Contractor's Project Director: Dr. Michael M. Sigel

Project Officer (NCI): Dr. Paul H. Levine and Dr. Clarice E. Gaylord

Objectives: (a) to determine, define and integrate the relevant factors in host defense against mammary tumors, (b) to analyze cellular immunity in relation to clinical state and (c) to compare lymphocyte responses to MTV-associated, MTV-induced and non-MTV tumor-specific antigens in mice with breast cancer.

Major Findings: The results of assays of lymphocyte responses to tumor antigens and mitogens in mice whose tumors had been removed surgically confirm and extend previous findings. The new results indicate a correlation between the immunologic status and the postsurgical fate of the animal (no tumor, minimal tumor or relapse with small, large or metastatic tumors).

Early passages of D1-DMBA-3 (I) and D1-DMBA-2 (N) tumors in MTV Balb/cfC_3H mice have not detectably altered their antigenic constitution. They appear not to have acquired (at least as yet) either MTV or MTV-associated antigens. I_{BFC} (I passed through Balb/cfC_3H) caused lymphocyte stimulation exactly like its prototype I; N_{BFC} (N passed through Balb/cfC_3H) failed to elicit immunologic sensitivity or lymphocytic responses like its prototype N.

Mice with spontaneous mammary tumor respond to the autologous antigen. This tumor antigen is non-crossreactive with the antigens of the chemically induced tumors. Assuming that the spontaneous tumor contains MTV, the results further substantiate previous findings that the Balb/cCrgl mice and their tumor (I) are free from MTV, at least in detectable levels.

3M KCl extracts produced blastogenic responses in immune lymphocytes which were of somewhat lower magnitude than those produced by extracts prepared by homogenization.

Migration-inhibition (MI) studies have produced results analogous to those obtained with blastogenic transformation tests. Mitogens caused MI of peritoneal exudate cells (PEC) from normal mice and mice bearing small and medium tumors. A lesser MI was seen with PEC from mice with large tumors. MI was also evoked by extracts from immunogenic tumors, but not from nonimmunogenic tumors, in PEC from tumor-bearing mice.

MTV+ milk and purified MTV preparations caused blastogenic transformation reactions in the lymphocytes from MTV- mice but not (or to a lesser extent) in the lymphocytes of MTV+ Balb/cfC_3H. The latter mice did develop lymphocytes responsive to antigens in milk after transplantation with spontaneous mammary tumors. Analogous results were obtained in MI tests

en PEC from MTV- Balb/cCrgl mice were compared with PEC from MTV+ Balb/cfC_3H mice. Again, positive reaction with the latter cells occurred, usually, after transplantation of spontaneous mammary tumors. The results suggest that the latter mice are tolerant to some MTV-associated antigens but not to others and that the MTV- Balb/cCrgl have lymphocytes sensitive to an antigenic component associated with MTV, possibly the same one that the Balb/cfC_3H mice are tolerant to. These findings are considered to be of major interest inasmuch as mammary tumor incidence is considerably higher in Balb/cfC_3H than in Balb/cCrgl.

T cells and B cells were separated from the spleens of normal and tumor-bearing mice. Thus far, the major difference has been an increase in the proportion of B cells with complement receptors in the tumor group.

Significance to Biomedical Research and the Program of the Institute: The murine mammary tumor viruses (MTV) have been shown to be etiologically related to breast cancer in mice and there is some evidence for similar viruses being present in humans. Because of the laboratory and clinical similarities between breast cancer in mice and in women, the present work is designed to obtain a clearer understanding of the immunological reactivity of the host to an array of MTV and tumor-related antigens. The approach is to identify various antigens and immunologic as well as non-immunologic elements governing the relationship and interactions of host, virus and tumor. This project will provide information relevant to both etiology and control of breast cancer in humans.

Proposed Course: The proposed course of this study is to continue the assessment of the immunologic status and response in mice with different virological and immunological backgrounds and to correlate these responses to various clinical stages of disease.

Date Contract Initiated: March 15, 1974

Current Annual Level: $115,014

MOUNT SINAI SCHOOL OF MEDICINE AND HOSPITAL (NO1-CP4-3225)

Title: Stimulation of Immunity to Virus-Associated and Tumor-Associated Antigens in Mouse Systems

Contractor's Project Directors: Dr. James F. Holland
Dr. J. George Bekesi

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To pursue a program of chemoimmunotherapy in lymphoma-bearing AKR mice using neuraminidase-treated cells; (2) to study the stimulation of immunity to virus- and tumor-specific antigens in successfully treated and control mice; (3) to examine the efficacy of allogeneic Gross leukemia virus-induced E_2G leukemia as an immunogen; (4) to study the effectiveness of maintenance therapy using compounds which show antiviral activity, e.g., adenine arabinoside, Palm O-araC,

Virazole, and other drug combinations which may suppress the induction of clonogenic lymphoma cells in the treated host; (4) to determine the function of the spleen in the reinduction of secondary lymphoma in AKR mice; (5) in collaboration with Dr. Oldstone of the Scripps Clinic and Research Foundation, to determine the antibody-antigen complexing in the sera and kidney of animals receiving chemotherapy alone and AKR mice treated with chemotherapy and immunotherapy; (6) to determine the viremia of AKR mice in the experimental protocol (in collaboration with Dr. Zimmerman at Litton Bionetics, Inc.), in order to elucidate the mechanism of action of neuraminidase-treated leukemic cells in AKR mice infected in utero with GMLV and carrying large amounts of the virus throughout their life.

Major Findings: When Virazole was tested for antiviral activity against Gross leukemia virus (MLV) in vitro by the plaque-reduction assay, an 80% inhibition of plaque formation was achieved with as low as 25 ug of Virazole per ml of culture medium. Experiments performed in clinically diagnosed leukemic AKR mice with Virazole alone showed no apparent anti-tumor effect. However, in experiments where the clinically diagnosed AKR mice received combination cytoreductive chemotherapy (the best protocols are Vincristine and Cytoxan + methylCCNU or Vincristine + Prednisone) followed by Virazole, there was a delay in the reappearance of viable clonogenic lymphoma cells as measured by the explantation-splenomegaly assay, and an increased survival time for these animals compared to ones receiving cytoreductive therapy alone.

Using the above experimental protocols, attempts were made to determine the relationship between AKR virus titer and the type and clinical efficacy of treatment. AKR viral titer was measured by XC focus assay in AKR mice using the extracts of short (few mm.) sections of mouse tail. The AKR mice with spontaneous lymphoma were initially treated by cytroreductive chemotherapy, followed by antiviral therapy (Virazole) or immunotherapy (neuraminidase-treated isologous cells). The results indicate that chemotherapy and Virazole, as well as chemotherapy and immunotherapy with neuraminidase-treated cells produced a noticeable decrease in the viral titer evident on the 13th day of treatment.

The effect of various Vibrio cholerae neuraminidase concentrations on the dye exclusion of leukemia L-1210 cells was examined by the trypan blue vital staining method. Tumor cells incubated in buffer alone maintained their viability quite well, and even after three hours incubation produced no tumor when inoculated in non-immunized mice. Tumor cells treated with 25 to 75 units neuraminidase showed no marked change in their permeability to trypan blue; yet 10^8 such treated cells produced no tumor when inoculated in normal syngeneic mice.

Quantification of humoral and cell-mediated immunity was made in mice multiply immunized with neuraminidase treated or x-irradiated L-1210 tumor cells at the time of the first challenge, e.g. 21 days after the last immunization. Fifteen days after a challenge with viable leukemia L 1210 cells immunized animals possessed a dose-dependent antibody titer and powerful cell-mediated immunity; 43.5% of target cells were lysed by immune

lymphocytes in mice refractory to 10^8 untreated leukemic cells. It is significant that mice multiply immunized with x-irradiated leukemic cells showed strong antibody titer against L 1210 cells, while exhibiting very low levels of cell-mediated immunity. This in vitro observation was further substantiated by the following experiments: incubation of 18 viable untreated leukemia L-1210 cells with sera or cells from immunized (neuraminidase-treated L-1210 cells) mice neutralized their leukemogenicity and protected recipient test animals against the disease.

Studies on the efficacy of immunity induced by treatment of the host with neuraminidase-treated cells, with cells treated with agents such as mitomycin-C, or with soluble tumor-associated antigens are underway. These studies are carried out in virus-positive animals known to have a high incidence of mammary tumors, and in virus-negative animals before or after their receiving transplants of mammary tumors from syngeneic animals. In both virus-positive and virus-negative animals, similar studies are in progress, before and after transplanting chemically induced tumors.

Significance to Biomedical Research and the Program of the Institute: The contractor has successfully explored the parameters of neuraminidase incubation of spontaneous and experimental tumor cells and established treatment protocols which lead to their increased immunogenicity. They have had success with combined chemotherapy and immunotherapy using neuraminidase-treated cells in the cure of animals with leukemia L-1210 in $DBA/_2$ hosts, 316 melanomas in BALB/c mice, and in the cure of a substantial number of AKR mice having Gross virus-induced autochthonous leukemia. Similar results were obtained with Gross virus-induced allogeneic E_2G leukemia in AKR (leukemic) mice. No other data exist concerning the successful use of chemoimmunotherapy with neuraminidase-treated cells in an autochthonous system. Their data indicate substantially greater resistance to challenge than in any other reported experimental leukemic system using other types of immunization (such as chemically-modified leukemic cells, irradiated leukemic cells, MER or BCG). The knowledge gained from the treatment of transplantable and spontaneous leukemia and mammary tumor with Vibrio cholerae neuraminidase, with and without chemotherapy, is highly relevant to man suffering from neoplastic disease. Information obtained during this contract is now beginning to be applied in a clinical study of persons suffering from neoplastic disease.

Proposed Course: Studies in the AKR system will continue with emphasis on improving survival and determining (in collaboration with Dr. Michael Oldstone) the role of viral antigen-antibody complexes in determining the outcome of disease. In addition to exploiting similar techniques in murine breast cancer systems, an attempt will be made to exploit the potential importance of virus repression by establishing an interferon-associated adjunctive therapy in treating H. saimiri-induced lymphoma.

Date Contract Initiated: August 6, 1973

Current Annual Level: $300,000

NEW YORK MEDICAL COLLEGE (NO1-CP3-3398)

Title: Immunologic Measurements as a Guide to the Behavior and Viral Etiology of Breast Cancer

Contractor's Project Director: Dr. Maurice M. Black

Project Officer (NCI): Dr. Clarice E. Gaylord

Objectives: (1) To attempt to identify tumor and virus specific antigens in human breast cancer tissues; (2) to correlate measurements of patient immunologic reactivity with clinicopathologic characteristics of the tumor tissue and the host; (3) to search for evidence of the role of a mammary tumor virus in human mammary carcinogenesis by immunologic procedures and molecular hybridization procedures using MMTV as a probe (in collaboration with Dr. Dan Moore); (4) to determine immunological responses of women to MMTV and immunogenic breast cancer tissue according to "risk" factors, viz., parity, exogenous estrogens, and family history of breast cancer.

Major Findings: The skin window procedure was used to test cellular hypersensitivity responses of breast cancer patients against cryostat sections of autologous breast cancer tissues. Positive responses to cryostat sections of autologous breast tissues were preferentially found among patients who were clinically free of disease at the time of testing and who had stage 0 or stage I disease at the time of mastectomy. Positive responses were also found among patients having positive L-RE responses to their cancers at the time of mastectomy. Serially followed patients who had positive skin window tests became negative in association with recurrent disease. Individual patients have shown repeatedly positive tests more than three years postoperatively.

The leukocyte migration procedure was used as a measure of cellular hypersensitivity responses of human leukocytes against human breast tissue and MMTV-containing RIII milk. Breast cancer patients' leukocytes which were responsive to RIII milk were simultaneously responsive to the majority of L-RE positive autologous and homologous breast cancer tissues, less regularly responsive to L-RE negative breast cancer tissues and rarely reactive to control breast tissues and murine milk devoid of MMTV.

Rabbits and guinea pigs immunized with purified MMTV have shown a well marked delayed type hypersensitivity response to MMTV and MMTV- containing milks of RIII, C3H, DBA, A and GR mice. They were nonresponsive to milk samples from RIIIf and Swiss mice and nonresponsive to 10^9 RLV or MPMV virions. However, sensitized animals have thus far shown no response to eluates of cryostat sections or KCl extracts of breast tissues (benign or malignant).

The discontinuous PAGE has been used to compare the proteins in eluates of benign and malignant breast tissues. Eluates of immunogenic (L-RE-positive) breast cancer tissues were found to contain a distinctive band which was lacking in the eluates of the normotypic breast tissues, of melanomas

and of nonimmunogenic (L-RE-negative) breast cancer tissues. Of particular interest was the finding that similar studies of MMTV proteins disclosed that viral proteins were located in the same region as the distinctive component. After SDS treatment, PAGE revealed that the MWs of two components in this distinctive region were 51,000 and 32,500. Both components were absent in eluates of melanoma, normotypic ductular hyperplasia and nonimmunogenic breast cancers. Further characterizations of the physiochemical and antigenic properties of the distinctive components are underway.

Using tissues supplied and classified by Dr. Black, Dr. Dan Moore and his associates at the Institute for Medical Research have performed studies on the homology of breast cancer RNA with genomic material of MMTV. The data suggest that MMTV homologous RNA is not found randomly in human breast cancer tissues but appears to be associated with family history of breast cancer, the in situ stage of disease, and high nuclear grades.

Significance to Biomedical Research and the Program of the Institute: Since the findings of this investigator strongly suggest that the in vivo and in vitro cellular hypersensitivity responses of breast cancer patients provide biologically significant indices of maximal tumor-host interactions in the initial stages of disease, the techniques used by the contractor may be useful tools in the possible early detection, diagnosis and effective treatment of breast cancer in women.

Proposed Course: During the next year the contractor plans: (1) to improve and expand technical procedures and expertise gained in prior studies with particular reference to correlations between clinico-pathologic, immunologic and biochemical measurements; (2) to characterize and evaluate cellular components of the in vitro hypersensitivity response in an effort to define optimal conditions for sensitivity and reproducibility; and (3) to establish banks of cryostat sections of precisely classified breast cancer tissues for use by other VCP investigators.

Date Contract Initiated: June 26, 1972

Current Annual Level: $134,353

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP5-3512)

Title: Immunopathologic Study of Leukemia

Contractor's Project Director: Dr. Michael B. A. Oldstone

Project Officer (NCI): Dr. Tadao Aoki

Objectives: (1) Identification and quantitation of viral antigens of Gross leukemia virus (GLV) and various antibodies to these antigens found in the virus-antiviral antibody (V-Ab) complexes of AKR/J mice. To develop quantitative, specific and sensitive assays for the detection of

antiviral antibodies in the presence of antigen excess; (2) to utilize this developed technology to study differences in V-Ab immune complex deposits between AKR/J mice spontaneously developing lymphoma and age-sex matched AKR/J mice treated with antiviral and anti-immune reagents.

Major Findings: By 8 months of age AKR/J mice of both sexes develop a greater than 80% incidence of immune complex deposits in the renal glomeruli. These complexes are recognized by staining the glomeruli of such mice with monospecific fluorescence conjugated antibody to mouse Ig and C3. Such deposited Ig can be immunochemically recovered by treating washed renal homogenates with low acid-low molar glycine-HCl buffer and removal of the majority of low molecular weight (less than 100,000 MW) viral antigens by filtration through an amnicon membrane and purifying the retained immunoglobulin (Ig) by rate zonal centrifugation. Such Ig specifically binds to radiolabeled AKR gross leukemia virus or p-30 when studied by rate zonal centrifugation. Preliminary analysis of radiolabeled Tween-disrupted oncornavirus (not GLV)-eluted Ig immune precipitate by SDS acrylamide gels indicated the presence of antibodies to p30 and p69-71.

Utilizing similar techniques in collaboration with George Klein it was found that kidneys from two patients with Burkitt's lymphoma had deposited immune complexes. Elution of the glomerular-bound Ig indicated that it reacted with VCA and EA but not with EBNA and MA. Other studies showed that 25% of the total glomerular eluted Ig was absorbed by cells expressing EBV antigens. Further studies showed the presence of complement binding complexes in the serum of selected patients with African Burkitt's lymphoma.

Significance to Biomedical Research and the Program of the Institute: Over the past several years, there has been intensive investigation of the immunopathogenesis of persistent viral infections and virus-induced immune complex disease. Of particular importance and application to the cancer field and to the program of the Institute are the contractor's observations that in persistent viral infections in which a low antibody response is made, viral-antiviral antibody complexes may be found in the circulation and subsequently become trapped in the renal glomeruli. Because of antigen (virus) excess, free antibody is not found in the circulation, but can be recovered, identified and quantitated in the renal glomeruli. The techniques used in the Principal Investigator's laboratory for analyzing the viral antigen-antiviral antibody complex have led to a convenient marker for identifying the persisting agent in the absence of detectable free antibody. This suggests that in looking for markers to identify the cause of human cancer, the techniques used in animal models of persistent infection should be applicable. Of prime importance in this regard is the observation that in spontaneous leukemia of AKR mice, antibody to Gross virus or Gross virus cell surface antigen can be found and recovered from the renal glomerulus where they have been deposited in the form of antigen-antibody immune complexes. Hence, direct immunologic evidence has been found for identifying the etiologic agent in spontaneous murine leukemia by immunochemical techniques, and possibly these procedures will be of importance in the study of spontaneous leukemia and cancer of man. Also,

the finding of complexes in patients with Burkitt's lymphoma suggests that monitoring these and similar patients with neoplasia may provide valuable markers for diagnosing etiologic agents in neoplasia and following serum for evidence of tumor recurrence.

Proposed Course: The research plan is to complete the identification of various antibodies to viral antigens in AKR mice and to utilize this knowledge to monitor the fate of V-AB complexes in AKR mouse with and without immuno- and antiviral therapy. This proposed experiment is to be done with Dr. Bekesi, Mount Sinai Hospital, who is actively treating AKR mice with various modalities of chemo and immunotherapy in an effort to alter the course of their naturally occurring spontaneous lymphoma.

Date Contract Initiated: September 1, 1972

Current Annual Level: $85,892

UNIVERSITY OF TENNESSEE (NO1-CP4-3325)

Title: Immunological Studies on the Relationship of Embryonic Antigens to Virus-Induced Tumor Antigens

Contractor's Project Director: Dr. Joseph H. Coggin

Project Officer (NCI): Dr. Gary Pearson

Objectives: The major objective of this contract is to apply immunological approaches to determine the relationship between virus-coded, tumor-specific transplantation antigens (TSTA) on the surface of cells transformed by oncodnaviruses and other transplantation antigens such as embryonic antigens coded for by the cellular genome.

Major Findings: (1) This study is designed to investigate three approaches to the problem of distinguishing between virus-coded, tumor-specific transplantation antigens (TSTA) on the surface of tumor cells transformed by oncodnaviruses and other transplantation rejection antigens coded for by the cellular genome. The first approach involves the use of cytotoxic, allogeneic sera prepared in goats against mid-gestation hamster fetal cells which are known to be positive for the expression of embryonic antigen (EA+). Absorption of the cytotoxic sera with term fetal cells removed non-specific cytotoxic activity against all hamster antigens which were non-EA+. Potent selected sera against hamster EA have been made over the past six months and have been characterized to have the desired cytotoxic selective potential sought. The characterization has involved screening against EA+ and EA- fetal cells and SV40 and adenovirus-induced tumor cells which were known to be EA+ and to carry an apparent TSTA as well which was believed to be unique to EA's reexpressed on the tumor cells. The sera are currently being used to select EA- TSTA+ variants from EA+ TSTA+ clones. The characterization of many of these selected clones is presently underway using the antibody (IAT, etc.) and cell-mediated assays (microcytoxicity, adoptive transfer, isotope release) to determine whether such lines can be

established and whether they maintain their antigenic traits in a heritable manner.

(2) Attempts to solubilize cell surface antigens from oncodnavirus tumor cells and from fetal cells have been concluded. Well characterized antigen extracts have been successfully prepared from fetal and tumor sources and their immunogenicity in vivo has been documented. IgG against the various preparations derived in syngeneic hamsters has been prepared and is currently being column purified in the cycling affinity chromatography system with excellent results. Antibody to extraneous antigens and calf serum have been eliminated. The biological characterization (e.g., capacity to induce tumor resistance) of the soluble antigens are nearly complete and the immunoelectrophoretic study to discern differences and similarities between purified EA preparations and antigens derived from tumor cells are underway. Difficulties caused by poor reproducibility with the microcytotoxicity test (MC) have recently been encountered and have been discovered to relate to the use of newly derived target cells in use following the 1973 hamster lymphoma epidemic. A modification of the technical procedures for performing the MC test and the use of an old stock of tumor target cells permits the contractor to proceed with the proposed desensitization experiments. Fetal and tumor antigens will alternately be used to desensitize effector cells from pregnant donors (EA sensitized) and tumor immune donors (EA and TSTA sensitized) to resolve the role of these antigens in tumor resistance and progression.

(3) Both soluble and cell-bound antigen preparations derived from tumor and fetal tissues are being examined for their concurrent reactivity against fetal cells in the fetal colony focus assay (FCFU test) in the third approach to distinguishing between EA and TSTA's present on viral tumor cells. The system has been successfully standardized for use in the mouse SV40 system.

(4) Lastly, a battery of isolation attempts are underway to isolate the agent responsible for the three hamster lymphoma epidemics encountered since 1972 in this laboratory. Immune responses to agent and tumor associated neoantigens are being characterized. The epidemiology of the agent and its relation to laboratory workers is being extensively studied. Attempts to demonstrate the absence or presence of EA on lymphomas appearing in the epidemic are being conducted in collaboration with the NCI.

Significance to Biomedical Research and the Program of the Institute: The hypothesis of depressed host genes being responsible for oncogenesis is being actively studied by several groups in cancer-related programs. The relationship between fetal antigens and viral-induced antigens is an important issue in relation to the etiology question as well as to programs concerned with prevention and control of virus-associated cancers. To better understand the mechanism of neoplastic transformation, it is essential to develop the tools to distinguish between host gene reexpression and new viral gene products.

Proposed Course: (1) Continue to attempt to select embryonic antigen (EA)-TSTA+ variants from EA+ TSTA+ clones using the cytotoxic serum containing antibodies to EA; (2) fetal and tumor antigens will alternately be used to desensitize effector cells from pregnant donors (EA sensitized) and tumor immune donors (EA and TSTA sensitized) to resolve the role of these 2 groups of antigens in tumor resistance and progression; and (3) to continue to test both soluble and cell-bound antigen preparations derived from tumor and fetal tissue for concurrent reactivity against fetal cells in the fetal colony focus assay(FCFU test).

Date Contract Initiated: June 28, 1974

Current Annual Level: $175,560

UNIVERSITY OT TEXAS (NO1-CP4-3370)

Title: Immunological Studies on Animal and Human Breast Cancer

Contractor's Project Director: Dr. James M. Bowen

Project Officer (NCI): Dr. Gary Pearson

Objectives: The major objectives of this contract are: (1) to apply a variety of immunological methods, both cellular and humoral, to detection and characterization of virus and tumor-associated antigens and their antibodies in mice with mammary cancer; (2) to apply similar methods to a study of antigens in cells derived from human breast cancer patients; and (3) to determine by these methods whether viruses similar or related to mouse mammary tumor virus may be associated with the human neoplasm.

Major Findings: (1) Initial studies utilizing various methods in comparative tests showed that mammary tumor-bearing mice produce antibodies against both mammary tumor virus (MMTV) antigens and non-viral antigens associated with tumor cells. The sera of tumor-bearing mice react with both cytoplasmic and cell-surface antigens of virus-producing mammary tumor cells, directly label budding and mature type B particles in ferritin-antibody tests, and precipitate intact type B particles in radioimmunoassays. Conversely, tumor-free mice of the same strains and ages were negative for these anti-bodies with very few exceptions.

(2) In assessing the presence of antibody in the many sera tested, there was generally good agreement between the results of immunofluorescence (FIF) and mixed hemadsorption (MHA) tests, and between ferritin-labeled antibody (FLA) and radioimmunoassay (RIA) tests. A significantly smaller number of sera were positive with the latter two tests.

(3) Sera of 39% of breast cancer patients tested and of 13% of their relatives gave positive FIF reactions with cells of a type B virus particle-producing mouse mammary tumor cell line. The reactions appeared due to a complex of heterophile, tissue, and MMTV-specific antibodies in the sera. Sera of a large number of normal donors were negative. The basis of these reactions is currently under study.

(4) New studies include further characterization of antigens in cells derived from human breast cancer, analysis of humoral cytotoxicity in tumor-bearing animals and in breast cancer patients, and analysis of the humoral role in tumor graft rejection. In addition, studies of the role of lymphoid cells in tumor graft rejection will be undertaken, in both mouse and cross-reacting systems.

Significance to Biomedical Research and the Program of the Institute: Mammary tumor viruses have been shown to be etiologically related to breast cancer in mice, and there is some evidence for similar viruses being present in humans. Because of the laboratory and clinical similarities between breast cancer in mice and humans, the present work is geared toward obtaining a clear understanding of the immunological reactivity to virus and tumor associated antigens, to identify viral-related antigens in breast tumors, and to relate these immune responses with the stage of disease. This approach is of direct significance to human breast cancer, since it involves an assessment of immunological parameters with the clinical course and with therapeutic interventions.

Proposed Course: This project aims at the application of various humoral and cell-mediated immunological methods to study the immune response of mice to their mammary tumors, of women to their breast cancers, and to the elucidation of immunologic cross-reactivity between mouse and human breast cancer. The role of viral antigens in the immune response is emphasized. Immunofluorescence, immuno-electron microscopy, immunodiffusion, radio-immunoassay and other immunochemical procedures will be employed to: (a) detect, in sera of mammary tumor-bearing and non-tumor-bearing mice, naturally occurring antibodies to mouse mammary tumor virus (MMTV) antigens and to other antigens associated with mammary tumor cells; (b) characterize the antigens (and their antibodies) with respect to their type (viral, tumor-associated, embryonic, heterophile), distribution, time of appearance, and titer as a function of the age of the animal and relation to appearance and progression of tumor; (c) detect and characterize a similar spectrum of antigens and antibodies to these antigens in sera of patients with breast cancer, their relatives and associates, and of patients with other types of tumors; (d) analyze in detail the demonstrated cross-reactivity between antibodies in sera of breast cancer patients and viral or tumor antigens associated with mouse mammary tumor cells in an attempt not only to demonstrate the presence of RNA tumor virus information in human breast cancer cells, but also to determine whether the putative human breast cancer virus is related to MMTV.

Date Contract Initiated: June 28, 1974

Current Annual Level: $105,881

UNIVERSITY OF TEXAS, M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CP3-3301)

Title: Oncogenic Virus-Associated Immunity and Immune Responses in Human Cancer

Contractor's Project Director: Dr. Evan M. Hersh

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To study the human immune response to the Rauscher leukemia virus (RLV), including studies of both cell-mediated and humoral immunity; (2) to generate reagents useful in studies of immunity to RLV antigens and the possible viral etiology of human neoplasms; and (3) to develop methodology for the possible viral immunotherapy of human cancer. This will ultimately involve immunization with oncogenic viruses or preparations derived from oncogenic virus-transformed cells for the treatment or prevention of recurrence of cancer.

Major Findings: Analysis of humoral and cell-mediated immunity assays were completed in the collaborative studies comparing formalinized Rauscher leukemia virus inoculated into humans, mice, and rhesus monkeys. Radioimmunoassays detected a significantly high specific antibody titer to RLV in all three groups. No cellular immunity response was demonstrated in the mice or monkeys, but in the humans a significant CMI response was detected by skin test and lymphocyte stimulation. A comparison of immune response to live and formalinized Rauscher leukemia virus (RLV) performed only in the mice and monkeys have demonstrated that the live virus yielded a much more vigorous immune response than the killed virus.

Antisera prepared in the successfully immunized humans and monkeys were utilized in the fixed immunofluorescence assay to detect antigens cross reacting with RLV in the bone marrow and peripheral bloods of human leukemia patients. The bone marrow was found to be a much better source of cross reacting antigens than the peripheral blood even though the peripheral blood frequently was filled with blasts.

Significance to Biomedical Research and the Program of the Institute: These studies may lead to the possible development of a vaccine with potential utility in acute leukemia. They will contribute materially to our understanding of the immune response to oncogenic RNA viruses and will have considerable applicability if it is determined that an RNA virus causes any form of human cancer. The finding that humans have a greater cellular immune response to formalinized RLV extract than mice or monkeys suggests that caution must be made in extrapolating experimental findings to human disease.

Proposed Course: This project will be terminated on May 23 because of the need to develop additional animal model systems to insure the safety and efficacy of future virus preparations and virus components in human studies.

Date Contract Initiated: May 25, 1972

Current Annual Level: $146,043

University of Texas (NO1-CP3-3292)

Title: Studies of Immunity to Sarcoma-Related Antigens in Patients and Controls

Contractor's Project Director: Dr. Joseph G. Sinkovics

Project Officer (NCI): Dr. Gary Pearson

Objectives: The overall major objective of this contract is the collection of evidence for human sarcoma-specific antigens as a means of identifying a human sarcoma virus. This has been approached through the following projects: (1) the establishment of human sarcoma cell cultures for immunological and virological investigation largely in collaboration with other contractors in the VCP; (2) long-term evaluation of the correlation between the clinical course of patients with sarcomas and immunological parameters; and (3) determination of the possible role of an infectious agent in the sensitization of lymphocytes from clinically normal, healthy individuals to long-term cultured sarcoma cell lines in the in vitro cytotoxicity assay.

Major Findings: (1) It is becoming quite apparent that normal, healthy donors are capable of displaying lymphocyte-mediated immune responses against tumor cells in vitro. During the past year, data were presented which indicated that, in a microplate assay, approximately half of the normal donors tested possessed lymphocytes significantly cytotoxic to cultured sarcoma cells, while approximately one-third reacted strongly to cultured carcinoma cells. Present data shows that when the larger LabTek assay is used, normal donors continue to show in vitro reactivity of lymphocytes to tumor cells, but not as frequently as had been observed in the microplate assay. Of 24 normal individuals evaluated, lymphocytes of 6 (25%) significantly reduced the number of sarcoma cells, while these same preparations remained unreactive to cultured carcinoma cells. No serum factors blocking cytotoxic lymphocytes were evident in these tests, however, in contrast to evidence found in the earlier microplate assay. When whole buffy coat leukocyte preparations were used in the LabTek wells, reactivity to tumor cells was frequent, but without distinctive specificity for tumor type. The discrepancy between microplate and LabTek chamber/slide data concerning the reactivity of normal donors will be resolved when the reactivities of the same individuals in both assays will be statistically evaluated.

(2) In studies involving cancer patients, preliminary data indicated a cross-reaction between sarcoma and melanoma but not between melanoma and carcinoma tissue culture cells.

(3) Among the recognized serum factors which alter cell-mediated immune reactions in vitro (such as blocking, potentiating, arming, etc.), another potential effect of sera might be "toxicity" of heat-inactivated sera to target cells. This would help to explain the recently observed high level of serum-enhanced target cell reduction in the presence of lymphocytes from both patients and normals, whether or not the lymphocytes themselves

displayed cytotoxic potential. It remains to be answered at this point whether or not the serum itself is toxic to the cells, or merely prevents viable cells from sticking to the glass. Preliminary data suggest that the latter situation may occur.

Significance to Biomedical Research and the Program of the Institute: The significance of this project is multiple: (1) identification of a common sarcoma-specific antigen would suggest the possibility of viral involvement in the etiology of sarcomas; (2) the observation that normal individuals possess circulating lymphocytes cytotoxic to sarcoma cells raises the possibility that a high percentage of normal individuals become exposed to an infectious agent which may produce sarcomas. Definition of the factors responsible for the sensitization of normal lymphocytes to sarcoma-related antigens could lead to the discovery of a human sarcoma virus; (3) this project may provide an in vitro assay capable of monitoring immune reactions of patients with sarcomas to their tumors and therefore may be useful in prognostication and evaluation of experimental immunotherapy protocols; and (4) established human sarcoma and other tumor cell lines will be available to interested investigators for virological and biochemical studies.

Proposed Course: In view of the apparent absence of tumor specificity in the human sarcoma system, the ongoing studies will be completed and analyzed in the current contract year. This contract will terminate on May 31, 1975.

Date Contract Initiated: April 1, 1971

Current Annual Level: $79,920

TULANE UNIVERSITY, DELTA REGIONAL PRIMATE RESEARCH CENTER (NO1-CP3-3396)

Title: Role of Humoral and Cellular Immunity in Determining the Outcome of Herpesvirus saimiri Infections in Squirrel Monkeys

Contractor's Project Director: Dr. William P. Allen

Project Officer (NCI): Dr. Dharam V. Ablashi

Objectives: The general purpose is to investigate the role that immunological factors may play in infections of Herpesvirus saimiri (HSV) in the squirrel monkey (Saimiri sciureus), its natural host. Specifically, the aims are: (1) to continue hand-rearing of HVS-free squirrel monkeys born in 1973 for eventual breeding stock; (2) to hand-rear some infants born in 1974 for purposes of breeding and others for experimental infection with HVS; (3) to determine the presence or absence of HVS antibodies, latent virus or viral genome in tissues of neonatal squirrel monkeys derived by natural birth or caesarian section from HVS-infected mothers; (4) to initiate studies on the effects of immunosuppressive agents on the outcome of HVS infection and the immune response in squirrel monkeys previously unexposed to the virus; (5) to characterize the primary antibody response to HVS in infant and juvenile squirrel monkeys experimentally inoculated with the virus.

Major Findings: The breeding colonies of squirrel monkeys at this center yielded 54 pregnancies and 30 births this year that have remained viable. Twenty-five of these infants have survived hand-rearing for one to three months and twelve of fifteen infants born in 1973 have survived one full year of hand-rearing. All females and several males are being held in isolation.

Experiments have been initiated to study the effects of immunosuppression with 16 mg/kg ALG given 3 times weekly to neonatal monkeys experimentally infected with HVS. All but 2 neonates had maternally-derived IgG antibody to late viral antibody (LA) and 1 had IgG antibody to early viral antibody (EA) at the time of virus inoculation. By day 36, virus was isolated from the blood of only 1 animal. Antibody responses to HVS in both suppressed and untreated newborns have been minimal and somewhat delayed as compared with responses in older animals. It is suggested that the presence of maternal antibody to HVS in newborns alters the course of infection and the immune response. An immunosuppression experiment has also been initiated with juvenile animals that have been reared in isolation for 1 year. None of the 6 animals in this experiment had detectable levels of antibody to HVS prior to experimental infection. Immunosuppressed animals developed a lymphocytopenia and infection with HVS after inoculation. HVS was isolated on several occasions from oral swabs of the immunosuppressed monkeys but not from blood lymphocytes. The reverse was observed in unsuppressed animals. One of 3 suppressed animals died on the 29th day presumably because of bone marrow depression by the cytotoxic drugs. No evidence of lymphoma was detected in the gross necropsy. Assays for humoral antibody in these animals revealed that the appearance of IgM and IgG antibody to HVS was delayed at least 2 weeks in 2 of 3 immunosuppressed monkeys. Antibody titers to EA and LA in the suppressed group continued to be lower than in the control group.

Observations on natural transmission of HVS revealed evidence of transplacental transmission of virus in only 1 of 3 stillborns and 8 newborns born to naturally infected mothers. This infected infant was born prematurely and died 3 weeks later. Apparently, HVS is not ordinarily transmitted transplacentally. Other observations indicated that one infant was reared by its naturally infected mother for at least 1 year without itself becoming infected.

Growth of HVS was compared in Callicebus skin (Cs) and owl monkey kidney (OMK) cells using uniform inocula and procedures. Growth curves revealed some striking differences. HVS grown in OMK cells appeared to be considerably more cell-bound than that grown in Cs cells, and peak titers of virus in the supernatant from Cs cultures were almost 100-fold greater than peak titers of supernatant from OMK cultures. In both cell cultures, maximum titers of HVS were attained between the fifth and sixth day. After the seventh day, titers declined sharply.

Significance to Biomedical Research and the Program of the Institute: The suggestion that viruses may induce neoplastic disease in man has loomed as one of the foremost biomedical research challenges in recent years. A major component of the evidence supporting this suggestion is represented by the association of Epstein-Barr virus (EBV) to Burkitt's lymphoma and other human lymphoproliferative diseases. There are several well-documented similarities between the natural history of EBV in man and HVS in squirrel monkeys. A most pressing question on the relationship of EBV and human cancer is the role played by the immune response. The squirrel monkey infected with HVS promises to provide an excellent model system to investigate many parameters of the immune response at both the humoral and cellular levels. The inability of EBV to elicit oncogenesis in most human beings, and a similar lack of tumorigenesis in squirrel monkeys infected with HVS, strongly suggests the need for a detailed investigation of immune mechanisms in both species. Many of these studies can only be done in a laboratory host system. The results could lead to greater insight into the relationship of viruses and human cancer.

Proposed Course: Current studies will be continued. Additional studies dealing with the effect of immunosuppression on HVS infection will involve the use of splenectomy, high doses of prednisolone, concanavalin A, and anti-IgM antibody. Cell-mediated immunity will be studied, utilizing the lymphocyte blastogenesis and ^{51}Cr release cytotoxic assays. Studies with possible natural antibody to HVS-induced lymphoma cells will be initiated. A considerable stress will be placed on further improvement of breeding colonies of squirrel monkeys with special emphasis on breeding, handling, special care for hand-reared infants, and better survival rate of newborns.

Date Contract Initiated: June 29, 1973

Current Annual Level: $96,299

UNIVERSITY OF WASHINGTON (NO1-CP3-33236)

Title: Immunological and Transplantation Studies on Dogs with Cancer for the Detection of an Oncogenic Virus Carrier State

Contractor's Project Director: Dr. Rainer Storb

Project Officer (NCI): Dr. Gary Pearson

Objectives: The major objectives of this contract are: (1) to carry out successful allogeneic bone marrow grafts in dogs with lymphoma to determine whether relapses occur in donor cells which could suggest a viral etiology in canine tumors; (2) to set up and assess the usefulness of immunological assays for demonstrating tumor-associated antigens on canine tumor cells; and (3) to carry out virological and biochemical studies for the detection of an oncogenic virus carrier state in dogs with lymphoma, breast cancer, osteogenic sarcoma and melanoma.

Major Findings: (1) During the past year a total of 106 dogs was referred to the institute of which 39 had lymphosarcoma (L) or leukemia, 43 had solid tumors (ST) and 24 either did not have malignant disease or were "biopsies only." Studies in these dogs involved immune reactivity studies, total body irradiation (TBI) and marrow grafting, attempts at cell free transmission of canine L, development of antibodies against tumor cells, demonstration of TAA, cytogenetic studies and attempts at determining a possible viral etiology of canine L.

(2) The humoral and cellular immune reactivity and reticuloendothelial clearance of dogs with spontaneously occurring malignancies were evaluated. Sixty-two dogs with malignant lymphoma or lymphoblastic leukemia (L) and 35 dogs with solid, non-hematologic malignancies (ST) were studied prior to any treatment. Studies of humoral immunity revealed significantly low IgG levels in dogs with L but not in those with ST. Hemagglutinin titers following sheep red blood cell immunization were suppressed in L dogs and normal in ST dogs. Similar findings were obtained following primary and secondary immunization with bacteriophage. Both groups of animals developed lymphocytotoxic antibodies following allogeneic skin grafts from unrelated dogs. Cellular immunity, assessed by first and second set allogeneic skin graft survival, was impaired in L dogs but not in ST dogs. Response to tuberculin challenge following sensitization with Bacille Calmette-Guerin and in vitro lymphocytic blastogenesis following stimulation by phytohemagglutinin or allogeneic lymphocytes was deficient in both groups of dogs. Reticuloendothelial function, assessed by clearance of bacteriophage from the circulation, was impaired in L dogs and normal in ST dogs.

(3) Utilizing standard techniques, attempts have been made to establish a number of canine lymphosarcoma cell lines in long-term tissue culture and have been successful in some instances. However, these cells did not grow logarithmically and there was a very high percentage of dead cells so that the number of living cells was remaining essentially stable. For these reasons, attempts to observe these cells for induction of virus particles after exposure to bromodeoxyuridine by EM have not yet been successful. More recently an exponentially growing cell line has been obtained. It is therefore planned to continue those studies with that particular cell line.

(4) Migration inhibitory reactivity to autochthonous tumor cells was tested in 24 dogs bearing spontaneous malignancies. The supernatants of mixed cultures of peripheral lymphocytes and autochthonous tumor cells were tested for ability to inhibit the migration of normal dog leukocytes. In 7 of 18 lymphoma dogs and 5 of 6 dogs with solid tumors, significant migration inhibitory reactivity was found. It was concluded that tumor cells from many dogs bearing spontaneous malignancies have tumor-associated antigens capable of stimulating the migration inhibitory reactivity of autochthonous peripheral lymphocytes in vitro.

(5) During the last two months of the reporting period dogs with lymphoma have been ultilized for allogeneic marrow grafts in order to obtain information on the value of this therapeutic approach in treating this disease.

In particular, it is hoped to obtain long-term survivors after transplantation which might serve as a model for the observed recurrence of leukemia in donor type cells observed in human patients with acute lymphoblastic leukemia.

Significance to Biomedical Research and the Program of the Institute: The dog with malignant disease is of particular interest as an experimental model for the continued study of spontaneous tumors in a randomly bred species. It is reasonable to suppose that principles derived from studies in such dogs may be more readily translated to human neoplastic disease than would be possible in studies of inbred animals or with induced or transplanted tumors. Evidence is being sought for the presence of an oncogenic virus in dogs with lymphosarcoma and breast carcinoma. Such evidence would be of special importance in understanding the relationship of tumors and virus--one of the major aspects of the Virus Cancer Program. Similarly, the nature and importance of tumor-specific antigens in malignant disease is a major focus of current investigations supported by the National Cancer Institute. It is planned to continue to explore a number of different assays for tumor-specific antigens, in an attempt to establish the incidence and importance of such antigens in naturally occurring dog tumors. Finally, m rrow transplantation is a current therapeutic approach to the treatment of human leukemia. One problem encountered in these clinical studies has been recurrence of leukemia in donor-type cells, which has been attributed to viral transformation. Studies are being conducted in tumor-bearing dogs in an attempt to observe this phenomenon in an animal model as an approach to the identification of an infectious agent in the etiology of canine malignancies.

Proposed Course: Future studies will be limited to those projects which can be funded under the grant system since the contract will terminate June 30, 1975. (1) Major emphasis will be placed on attempts to define the optimal conditions for successful allogeneic marrow grafting in dogs with cancer so that the question on the recurrence of malignancy in donor cells can be pursued; (2) studies on the existence of tumor-associated antigens in canine tumors will be continued using serological and cell-mediated immunity assays; and (3) attempts to recover an endogenous canine virus by co-cultivation or induction procedures will continue.

Date Contract Initiated: November 1, 1971

Current Annual Level: $93,335

SUMMARY REPORT

4. SOLID TUMOR VIRUS SEGMENT

July 1, 1974 through June 30, 1975

The Solid Tumor Virus Segment contract program functions in a highly interdependent collaborative manner to meet the following objectives:

1. Determination of the specific role of RNA and DNA viruses in the etiology of cancer, alone and in combination with chemical and physical carcinogens. The viruses under study include those transmitted endogenously by genetic inheritance and those transmitted exogenously, either vertically or horizontally. Primary emphasis is on those viruses which occur naturally and which are known to be etiologically associated with spontaneous cancers in the host species.

2. Identification at the cellular level of specific host gene control factors, such as regulating mechanisms which either predispose to increased or decreased RNA virus and tumor expression and their correlation with natural and chemically induced cancers.

3. Studies of the specific host immune response factors governing permissive or regulatory control over virus and/or cancer expression. The immune responses elucidated will be analyzed to identify those factors which can be used in the development as vaccines or other virus-inhibiting products to prevent or control cancer.

Studies of exogenous environmental factors which are known to induce endogenous oncogenic virus expression and subsequently cancer in a number of species. Emphasis is placed on carcinogenic potential for switching on endogenous virus expression and the use of virus positive or viral-infected cell systems for carcinogenic screening of environmental substances, including air pollutants, food additives, drugs, etc.

Major Accomplishments

Marked progress has been made in defining the genetic factors governing RNA virus expression and regulation, and cancer incidence. Dr. Frank Lilly (Einstein, #33249) has defined the gene complexes (Fv-1 and MuLV-1) which serve as regulators of oncornavirus expression and regulation in inbred mice, and more importantly has identified and characterized structural virogenes and determined their chromosomal loci. Some of the gene products governing cancer virus inhibition have already been tested by Drs. R. Tennant and A. Brown.

Dr. Hans Meier's group (Jackson, #33255) demonstrated that the expression of endogenous type C RNA tumor virus is under the control of two genes in an AKR x C57L cross, the occurrence of tumors is predictable, and correlates

quantitatively with virus expression, thus showing that the endogenous viral genes are oncogenic. In addition, they have discovered four genes affecting virus replication and tumor incidence in addition to those described by Dr. Lilly. They are currently defining the relationship of virus expression and tumors with various host marker genes. Dr. Meier's group is developing a number of recombinant inbred lines which will be particularly valuable for use in testing the carcinogenic potential of carcinogens and cancer vaccines, where the effect of such products can be ascertained in relation to a variety of cancer-susceptible and resistant murine hosts. These studies have direct applicability to current tests on the effect of vaccines in prevention of cancer in inbred mice and for in vitro and in vivo tests of suspected carcinogens. These studies in well understood model systems should prove invaluable when similar undertakings are posssible in higher mammals, including eventually humans.

Dr. L. Crittenden,(USDA #40214) has evidence suggesting that while nearly all chicken embryos produced RAV-0, the endogenous type C virus of the chicken, control of its spontaneous production post-embryonically is controlled by a dominant gene. He demonstrated further that RAV-0 is very inefficient at inducing lymphoid leukosis compared with RAV-1, a known leukosis-producing agent, but studies have not been on long enough to determine its long-term oncogenic potential which would be more analogous to the natural situation; most cancers in natural species occur in later life.

The major emphasis within the contract program is the definition of the role of the endogenous type C RNA viruses in naturally occurring cancers. Dr. Jay Levy's discovery of the xenotropic viruses which are part of the host cell genome and will not infect host cells was a major finding in FY 1975. The subsequent discovery by Dr. Stuart Aaronson's group (NCI) of natural antibodies to the xenotropic viruses was another important step in the search for the human type C virus and now provides logical approaches to control in the natural host. Dr. Jay Levy (U. California, #43381) is now adapting the procedures used in uncovering the murine xenotropic viruses to human materials. He has established a number of cell lines from selected sites derived from lupus erythematosus patients, which in turn will be co-cultivated with other cell lines, and treated with viral inducing agents.

Similar efforts to grow material from immunodefective people for the purpose of rescuing a human virus are underway at the Scripps Clinic and Research Foundation (#43375). Placentae from humans with lupus erythematosus have been shown to contain type C particles, and on co-cultivation with primate or rat fibroblasts generate reverse transcriptase activity and endogenous virus formation. Intensive efforts are now underway to get the endogenous virus to grow in a susceptible cell so that it can be produced in quantity. In addition, Dr. Lerner and others in Dr. Dixon's group have found that the GIX antigen of murine thymocytes, known to be a genetically determined alloantigen of the mouse, is identical to the 69-71 glycoprotein of murine leukemia virus (MuLV),thus supporting the concept that the MuLV genome represents classically inherited mendelian genes. This group is now engaged in isolation of the

gp 69-71 from several murine leukemia viruses (RLV, SLV, and xenotropic) to develop specific immunologic reagents in order to determine the precise role of different viruses in the oncogenic and immunologic diseases of various mouse strains. They have found tissue of the male genital tract to be a rich source of gp 69-71 in virtually all strains of mice tested.

Dr. Henry Kaplan (Stanford, #43228), has succeeded in establishing cell lines from a number of patients with diffuse histiocytic lymphomas, an American Burkitt lymphoma, A B-lymphocyte lymphoma and Sézary syndrome variant of mycosis fungoides. The histiocytic lymphoma cell lines and one American Burkitt lymphoma line are negative for the Epstein-Barr Virus (EBV) genome, despite the fact that some of the patients from which they were derived had EBV-positive serologies. Using the indirect immunofluorescence test, Dr. Kaplan's group has evidence suggesting that the histiocytic lymphoma cells bear a common (tumor-specific or carcinoembryonic) antigen. These lines have been successfully transplanted into the brains of nude mice, and will be used for a host of immunologic and biochemical studies, as well as prime sources for induction of a human type C virus. This program has been in effect for less than a year.

One of the most important assays in defining new viral isolates is the radioimmune test. Perhaps the most accomplished laboratory in the contract program for identifying viral isolates is Flow Laboratories (#43388). Dr. Gilden's group has created a "family tree" of genetically related oncornaviruses which involves several classes and species. Because of the high specificity of Gilden's reagents, his group has been able to quickly recognize bona fide new isolates as well as viral contaminants. One striking finding has been the very close interspecies relationship of baboon and RD114 p30s. Partial relationships have been demonstrated with other proteins and assays of other viruses. Based on Gilden's finds and those of other NCI scientists (Sherr, Benveniste, Todaro), extensive attempts to detect p30 determinants using a variety of direct and indirect immunoassays for p10, 12, 15, 30 and 70 in human tumors are in process. p15 (or one of several components in this size range) and p70 appear to be surface components which promise to be important for development of subunit vaccines in which virogenes would be discarded and protein immunizing subunits utilized for protective vaccines, thus eliminating aberrant potentially dangerous viral RNA and DNA. They have also identified a possible precursor to the low molecular weight polypeptides in mouse L-cells. In addition to reagents produced on site, Dr. G. Tregear (Harvard) has provided quantities of synthetic peptides developed on contract #33265 for use in the development of the specific radioimmunoassay procedure used in genetic mapping of p30 polypeptides.

Molecular hybridization studies in the Flow Laboratory, Inc., program have established a high degree of species specificity for mouse, hamster and rat endogenous viral genomes. Through its use, Dr. Gilden's group has found that viruses passaged through heterologous species lines, however briefly, pick up virogenes and proteins of the new species cells involved. Thus hamster viruses passaged through mouse and rat lines contain not only rat, mouse and

hamster viral proteins but have RNA sequences of mouse, rat, and hamster as well. This has helped explain problems created by the lack of agreement between immunological and hybridization results unless the virus passage history is known.

The gibbon virus was shown to cross-hybridize with that of the hamster as well as to the previously reported cross reaction with mouse viruses. This raises the question, based on immunologic uniqueness, whether this and the woolly monkey virus are not examples of viruses which have undergone reassortment under natural conditions.

Much of the methodology as well as the reagents responsible for the original scientific findings on this contract and in many other laboratories working on related problems were developed under this contract.

Microbiological Associates (MAI), on contract #33248, has been focusing its efforts on immunoprevention of spontaneous neoplasms in animal model systems that have viral and epidemiological similarities to human neoplasia. To this end, they have developed a number of assays for cell-mediated immunity to type C viruses (delayed hypersensitivity "footpad" test, cell-mediated cytotoxicity test and a micro-adapted version of the lymphocyte transformation assay). Large scale tests with selected live and attenuated vaccines are now underway. Animals on test will be maintained for long periods of time sufficient to evaluate vaccines over their lifespan.

In addition to the on site vaccine program, the contractor for many years has furnished histological services to VCB and VCP projects. Over 15,000 histologic slides were examined during the contract year.

Drs. Karl and Ingegaard Hellstrom (U. Washington, #33372) are engaged in defining cytotoxic blocking and unblocking serum factors associated with tumor development and inhibition with embryonic tumor antigens, and with in vitro sensitization of lymphocytes to tumor antigens. They recently reported that lymph node and spleen cells from pregnant mice were found to be cytotoxic to cultivated syngeneic tumor cells; this effect was decreased by treatment with anti-serum and complement. It could be blocked by serum from pregnant mice and from mice with chemically induced sarcomas. The blocking factors were removed by absorption with neoplastic cells taken directly from mice or from culture. The blocking serum activity was found to persist in mice whose tumors had been removed 8-11 days previously and which had transplantation resistance against the tumors' unique antigens. Thus embryonic tumor antigens could induce a strong blocking activity, which remained at the time when blocking activity against unique tumor transplantation antigens were lost, suggesting that the blocking activity may be at least partially responsible for the common failure of embryonic antigens to act as transplantation antigens in vivo. Spleen cells from tumor-bearing mice were also shown to contain blocking factors.

Employing a well established in vitro rat cell system (clone H43) chronically infected with Rauscher leukemia virus (RVL), Dr. Paul Price and Dr. Aaron Freeman (Microbiological Associates, #43240) tested 10 food colors and two drugs, adriamycin and tofranil, for transforming and carcinogenic effects. Five food colors - two green, two red and one blue - and both drugs transformed the rat cells within a few subcultures, and all the transformed cells were carcinogenic after transplantation into newborn rats. Five other food colors failed to transform cells nor did the cells transplant in vivo.

The 10 food color dyes were also injected into newborn hamsters, mice and rats. At present writing three of the 5 colors have produced early lymphomas, but only in hamsters; thus a report on these new findings must await completion of the animal studies.

Drs. Price and Freeman have also established the critical role of oncornavirus expression in virus-chemical co-carcinogenesis by the prevention of 3-methylcholanthrene-induced rat cell transformation by prior treatment of H43 rat cells chronically infected with RLV with immune globulin to RLV. The specific anti-Igg added to the media also significantly reduced infectious RLV in the cell cultures. Normal control rat antisera and antisera to antigenically heterologous murine leukemia viruses failed to protect against transformation and oncogenesis.

Recently Dr. Wallace Rowe observed early spontaneous transformation (6-7 subcultures) in four H43 rat cell clones chronically infected with C57L/J xenotropic virus, suggesting that this strain of xenotropic viruses may have intrinsic transforming capabilities.

Dr. Freeman has developed a procedure for growing normal human skin cells on pig skin in culture. He is now adapting this procedure in attempts to establish human epithelial cells in culture for transformation studies. Preliminary results are encouraging but not as yet conclusive. The pig skin substrate is also currently being utilized in attempts to grow epithelial cells of various animal origins in the hope that chemically induced in vitro transformation of epithelial lines can be regularly accomplished.

Dr. Johng Rhim and his associates (Microbiological Associates, #53519) succeeded in establishing completely virus-free clones of a human cell culture containing a non-producer (NP) Ki-MSV genome. A similar Ki-MSV (NP) horse cell line was also established. Both the human and horse cells are susceptible to infection with a wide variety of oncornaviruses and are thus very useful, particularly for producing pseudotype sarcoma viruses. Pseudotypes have been made of the xenotropic, ecotropic and wild mouse viruses, the xenotropic baboon virus, and the exogenous horizontally spread gibbon viruses.

Extensive attempts to activate endogenous viruses in the Ki-MSV NP human cells and in placental tissue have failed in the sense that infectious virus has not been established in cultures, although type C RNA viruses have been seen in human placental tissues. Similarly, actively growing cultures treated with IDU, dexamethasone and insulin failed to yield infectious virus particles despite the fact that brief and marginal reactions have been found in tests for reverse transcriptase.

Dr. John Parker, with Drs. R. Huebner and K. Takemoto, confirmed the presence of polyoma T antigen in the University of Tennessee epidemic of hamster lymphomas. The horizontally transmitted University of Tennessee virus, however, appears not to be polyoma but a related papovavirus of hamsters described by Dr. A. Graffi in 1968. While the virus shares the T antigen of polyoma, the virion antigen is distinct.

The ongoing studies by Drs. Murray Gardner, Brian Henderson, Vaclav Klement and their associates (USC, #53500) of oncornavirus expression in switched on and switched off wild mouse populations in Los Angeles County led to four major findings: (1) As with inbred laboratory mice, the prevalence and titers of gs antigen (p30) expression in mouse spleen were directly correlated with the incidence of lymphoma and AML-like paralysis during the first year of life and with carcinomas later in life in a strain (Lake Casitas) of mice highly switched on for type C virus expression. (2) Transmission and specific neutralization of the high titered viruses isolated from the Lake Casitas mouse population furnished additional direct evidence that the endogenous oncornaviruses were responsible for both. (3) Prevention of virus replication, lymphomas and paralysis in the Lake Casitas colony was also achieved at the genetic level by cross-breeding with C57Bl/10Sn inbred mice, a strain that carries homozygous dominant genes for repression of the p30 and virion expression of wild type MuLV's. (4) The wild mouse viral isolates were shown to be amphitropic, having properties and host ranges of both ecotropic and xenotropic viruses.

Most rat oncornaviruses of the rat have been shown to have the properties of xenotropic viruses. However, studies of rat viruses by Drs. Rasheed and Klement resulted in several ecotropic isolations from Sprague Dawley and Fisher rat cells.

At the Childrens Hospital of Los Angeles, under subcontract with the USC, Dr. McAllister and his associates achieved successful transfection of the DNA from RD114, FeLV and GaLV viruses into RD cells. This was achieved in collaboration with Dr. Norman Davidson (Cal. Tech., #33242). Working together, these groups established the presence of proviral DNA in the infected cells. During the course of these studies, the molecular structure of the RD114 genome was defined. Since only 50 ngms of DNA was required to induce infection, attempts are underway to transfect NP indicator cells with DNA from human leukemia, sarcoma and other tumor cells.

Epidemiological and surveillance studies of human cancer in Los Angeles (Henderson, Pike, Mack and Gardner) led to several important accomplishments: (1) The cancer surveillance program now embraces 98% of all cancers occurring each year in Los Angeles, representing the collaboration of 185 hospitals. (2) Using available surveillance data, it was found (a) that the familial risk of breast cancer is apparently related to a genetic abnormality in hormone excretion; (b) that postulated maternal and milk transmission factors do not influence the incidence of breast cancer, thus suggesting that horizontal transmission of a mammary cancer virus is not responsible for breast cancer; and (c) significantly increased rate of death due to lung cancer in Caucasian

males was associated exclusively with a South Central area of Los Angeles, wherein a striking increase in airborne polycyclic aromatic hydrocarbons is regularly present. The latter observation was derived from several years of air sampling at 39 stations in Los Angeles County.

Drs. Edwin Lennette and John Riggs (California State Department of Health, #43209), in collaboration with Dr. Paul Arnstein (NCI) have initiated several vaccine test systems. They found that experimental immunizations of Swiss mice, using Swiss lymphoma cells expressing xenotropic type C virus on the cell surface as live and inactivated vaccines, provided significant protection against virus-free immunogenic sarcoma cells. The protection appeared to be the result of specific cell-mediated immune responses rather than humoral in origin.

Additional studies revealed that killed corynebacterial preparations augmented protection better than other adjuvants. Of particular interest was the finding that 3-methylcholanthrene-induced sarcomas were delayed and reduced in incidence by immunization with live type C virus-infected cell vaccines augmented by corynebacterial preparations.

Drs. Lennette, Riggs and Oshiro described papovaviruses in a number of human laryngeal papillomas and genital warts. Cultures established from these papillomas have been successfully established. However, neither papovaviruses nor type C viruses have as yet been observed.

Dr. Michael Bishop's group (U. California, #33293), using molecular hybridization techniques, reported the very interesting finding that DNA's of mouse mammary tumor virus (MMTV) from different strains of mice contain different amounts of MMTV-specific nucleotide sequences. This was accomplished with the use of a restriction endonuclease. Thus, contrary to previous concepts, the MMTV's have diverged significantly in different strains of mice, a significant observation derived by molecular studies which conforms with observations on the natural history of the various mammary tumor viruses.

Bishop's group also found that proviral DNA for MMTV appears to occur only in the genus Mus. However, RNA from two of 40 human breast tumors showed appreciable homology with the MMTV genome, but contrary to other reports no homology was found with human DNA and MMTV DNA.

Bishop's studies of five sarcoma viruses and five different leukemia viruses of the chicken revealed the regular absence in the latter of genetic material (RNA-DNA) present in the sarcoma viruses. It was postulated that the missing sequences in the leukemia viruses could be due to the absence of the unique sarcoma genome sequences. Current studies are designed to clearly answer this question.

Dr. Maurice Green and his associates (St. Louis U., #43359), report new methods for labeling adenovirus and herpes virus DNA, and restriction fragments associated with transformation in vitro. Eleven adenovirus 2 DNA positives were found in analyses of 61 human gastrointestinal and lung tumors; of 28 normal tissues four were also positive, but two were from cancer patients and two from patients with diverticulosis.

Dr. Green also identified a subclass of early adenovirus 2 genes involved in transformation. During studies of early events in this infection, a 75,000 molecular weight early protein was isolated which binds to single stranded DNA and has the properties of T antigen. Radioimmune assay tests are being used to study the possible role of this protein in transformed cells and in human cancers.

Using a specific template primer, Dr. Green reports reverse transcriptase at densities of 1.16 g/cc in 50% of human breast cancers, and in 25 to 50% of a smaller number of human ovary carcinomas, Hodgkins disease and lymphomas.

In collaboration with Dr. Vogt (USC, #53518), Dr. Norman Davidson (Cal. Tech., #43306) showed that the molecules extracted from the transformation-defective mutant viruses have a molecular weight about 15% lower than that of the transformation-positive normal Rous sarcoma virus (RSV). This estimate, made from electron microscopy molecular weight measurements, was consistent with measurements in gel electrophoresis by Duesberg and Vogt.

Similar measurements of RD114, a xenotropic feline virus growing in a human cell, has about 60% more RNA information than do RSV and FeLV viruses. It is not clear how this might be reflected in unique but characteristic T or Y-shaped secondary structural features close to the middle of the RNA molecule visualized in the electron microscope.

Dr. Davidson has also collaborated with Drs. McAllister and Nicolson in successful transfection to RD cells of RD114 and FeLV viral DNA.

Dr. Peter Vogt's group (USC, #53518) has isolated 21 new temperature-sensitive avian mutants in the past six months. Several avian sarcoma virus ts mutants were fused to transform mammalian cells; the latter will now be used to test for complementation of transforming functions in the absence of virus synthesis. In collaboration with Dr. E. Scolnick (NCI), NRK cells transformed by ts mutants of murine sarcoma viruses will be included in these complementation studies.

Several multiple mutants have been segregated into single mutants by recombination. Of particular interest among the single mutants is one affecting an early transforming gene and two replication mutants, one in RNA synthesis, the other interfering with virus assembly and budding.

Dr. Thomas Kawakami (U. California, Davis, #33242) isolated three new gibbon type C viruses from clinically normal gibbons. Although identical in other respects, particularly in neutralization tests, collaborative studies with Drs. Aaronson and Stephenson (NCI) revealed that each isolate differed with respect to its p12 antigen. The gibbon virus (GaLV) is known to be transmissible horizontally to other gibbons, in some cases resulting in specific immune responses, and in others in a low level viremia.

Experimental infection of a young gibbon with bone marrow of a GaLV-infected gibbon led to a fatal myeloproliferative leukemia 13 months later.

Collaborative studies with Todaro and Gallo led to tentative information indicating that human myelogenous leukemia cells share molecular and antigenic moieties with the gibbon. Unfortunately the antisera used in these studies were made in heterologous animals, and currently available antisera from infected gibbons are in short supply and/or are non-reactive with cells of human myelogenous leukemia.

Dr. Leonard Hayflick (Stanford, #43244) reported transformation of normal human cells with chemical carcinogens. He has succeeded for the first time in transforming a normal liver cell culture to a malignant population using the chemical carcinogen 4-nitroquinoline 1-oxide (4NQO). This should now provide unique opportunities to study the mechanism of chemical carcinogenesis in human cells.

Dr. Hayflick has also developed a method for detecting HL-A specificities on cultured human cells, and has assessed their behavior in a variety of fusions between cytoplasts and whole cells derived from normal and malignant progenitors and as a result of transformation of normal human cells with the chemical carcinogen 4NQO.

Drs. Karl and Ingegerd Hellström, University of Washington, have demonstrated cytotoxic lymphocyte effect and blocking serum activity in pregnant mice. Thus, serum from pregnant mice could "arm" control lymph node cells and produce a lymphocyte-dependent cytotoxic effect on neoplastic but not on control target cells. They demonstrated further that "embryonic" tumor antigens elicited high titered blocking serum factors, and that mice with transplantation resistance to chemically induced sarcomas often had blocking factors to embryonic antigens. The large volume of data developed in several systems supports the following conclusions: (1) the poor ability of embryonic antigens to evoke tumor-specific transplantation resistance is due to their great capacity to form and act as blocking factors, abrogating lymphocyte-mediated, cytotoxic reactions; (2) transplantation tolerance may be maintained by an active immunological process and does not occur solely as the result of a deletion or irreversible suppression of potentially reactive lymphoid cells during neonatal life; (3) allograft tolerance may represent a defect of immune amplification. Generally, work during the past contract year supports the Hellströms' observations that serum blocking factors may play an important role in facilitating tumor growth in vivo and that the ability of tumors to induce the formation of such factors may be of fundamental importance for their escape from immunologic control. This evidence is further strengthened by the observation that "unblocking" antibodies are often found in animals whose tumors had regressed.

Dr. Robert J. Huebner, VCB, Division of Cancer Cause and Prevention, Chairman
Dr. James T. Duff, VCB, Division of Cancer Cause and Prevention, Vice-Chairman

ALBERT EINSTEIN MEDICAL COLLEGE (NO1-CP3-3249)

Title: Genetic and Immunological Factors in Viral Leukemogenesis

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To gain a better understanding of the genetic events and underlying mechanisms that play a role in the oncogenic process.

Major Findings: Studies of the mechanism of Fv-2 resistance to Friend virus (FV). Fv-2^r-homozygous mice are totally resistant to spleen focus induction at 9 days after administration of FV. Comparison of Fv-2-susceptible DBA/2 mice with partially congenic D2-Fv-2^r mice indicate that Fv-2^r inhibits the replication of spleen focus-forming virus (SFFV) by a factor of 30-100-fold (as contrasted with the much larger differentials suggested by earlier work using non-congenic animals). SFFV also generates transformed cells in the spleens of D2-Fv-2^r mice, although less efficiently than in DBA/2 mice. An important observation is that these tumor cells tend to grow diffusely in D2-Fv-2^r spleens, rather than in colonies as in DBA/2 spleens. (The expression of normal hemopoietic colony-forming cells in the spleens of lethally irradiated D2-Fv-2^r mice is not inhibited.) Although resistant to spleen focus formation and to early splenomegaly following FV, D2-Fv-2^r mice do eventually develop large spleens, polycythemia and die. It appears that several mechanisms may be involved in Fv-2^r-mediated resistance to FV, so that a common denominator has not been determined.

Fluctuation analysis of N-NB-tropism conversion. The use of Luria's "fluctuation test" to analyze the conversion of N-tropic FV to NB-tropism is currently in progress. Preliminary findings have not yet shown any evidence for the selection of a pre-existing NB-tropic variant as the mechanism of this conversion. The possibility that the conversion to NB-tropism may occur only after passage in the Fv-1-restrictive host is now being tested.

Studies of the mechanism of H-2-associated leukemia resistance. Studies of similarities and differences between FV-induced tumor cell lines derived from BALB/c (H-2^d) and congenic BALB-H-2^b mice were continued. Comparable cell lines derived from congenic H-2^k, H-2^g and heterozygous H-$2^{b/d}$, H-$2^{k/b}$ and H-$2^{g/d}$ mice are being developed and tested. Preliminary studies indicate that H-2^g cells resemble H-2^b cells in being virus producers (rather than nonproducers like H-2^d cells), but that the cells grow somewhat better in

vivo than H-2^b cells, i.e., they are less readily rejected by syngeneic hosts. The H-2^k cell line appears to be a nonproducer cell line, like H-2^d cells, but syngeneic hosts vigorously resist the growth of these H-2^k cells. Analysis of these results suggests a tentative interpretation: the K end of the H-2 complex influences the growth of FV-transformed cells (perhaps immunologically), whereas the D end of the complex governs virus replication by an unknown mechanism. Transformation of normal BALB-H-2^b spleen cells by FV was obtained in vitro, and the resulting cell line appears to resemble the BALB-H-2^b cell line obtained from a tumor induced in vivo, but this in vitro transformation has not been repeated.

Significance to Biomedical Research and the Program of the Institute: One of the basic facts about tumor biology is that genetic mechanisms of the host exert major control over the expression of oncogenicity. By defining the loci and markers associated with leukemogenesis, it should be possible to undertake systematic studies of the precise immunochemical mechanisms governed by individual loci, with the objective eventually of encouraging or altering their immunogenetic effects to provide maximum resistance against cancer.

Proposed Course: The contract will focus on the following problems: 1) the search for new genes which influence spontaneous and induced leukemia in mice, 2) studies of the mechanisms whereby known genes exert their effect, and 3) analysis of the natural history of the disease by means of this "genetic dissection".

Date Contract Initiated: May 13, 1965

Current Contract Level: $269,559

CALIFORNIA INSTITUTE OF TECHNOLOGY (NO1-CP4-3306)

Title: Electron Microscopic Studies of Tumor Virus Nucleic Acids

Contractor's Project Director: Dr. Norman Davidson

Project Officer (NCI): Dr. Edward M. Scolnick

Objectives: The contractor will apply physical methods, especially electron microscopy, to investigate the sequences and structures present in the nucleic acid molecules of RNA tumor viruses and in the integrated DNA viral genomes of transformed cells. Emphasis will be placed on the 35S constituents of the 70S RNA complex extracted from tumor viruses to determine molecular lengths, positions of poly-A sequences, and to determine if the 35S subunits are identical. Efforts will be made to isolate or enrich the small fraction of host DNA strands of transformed cells that bear integrated viral genomes. This will be done by hybridizing single-stranded DNA preparations with RNA bound to a solid support. By studying the structure of an RNA-integrated viral DNA hybrid in the electron microscope, it will be possible to determine the order of the sequences present in the integrated state.

Major Findings: During this first phase of this project, the following topics were studied:

1. Length measurements of the large subunits extracted from the following tumor virus RNAs:

   (a) Rous sarcoma virus (RSV) and transformation defective variants of RSV
   (b) Feline leukemia virus (FeLV)
   (c) RD-114

2. Nature of the secondary structure features present in RD-114 RNA.

Two methods of electron microscopy were used in these studies: 1) The glyoxal-formamide method developed by this laboratory which is particularly useful for breaking up hydrogen bonding interactions in a polynucleotide chain and giving well spread denatured molecules for contour length measurements, and 2) The urea-formamide method which is useful for extending those parts of RNA molecules which do not have any stable hydrogen bonding regions but at the same time maintaining the stability of certain kinds of stable secondary structure features.

The studies of the RNA of RSV and its transformation defective variant were conducted in collaboration with Dr. Peter Vogt. The observed length of the RNA of RSV corresponds to a molecular weight of $3.3 \pm 0.2 \times 10^6$ daltons. The molecules extracted from the transformation defective virus have a molecular weight of about 15% less. This reduction in molecular weight is consistent with measurements by gel electrophoresis by Duesberg and Vogt.

The molecular length of the RNA from the 35S subunit of the Gardner-Arnstein strains of FeLV was also found to have a molecular weight of $3.3 \pm 0.2 \times 10^6$ daltons.

Extensive studies were made of the properties of the RNA of RD-114 virus in collaboration with M. O. Nicolson and R. M. McAllister of the Department of Pediatrics, University of Southern California School of Medicine, Childrens Hospital of Los Angeles. The first surprising and unexpected result is that the length of the "35S" subunit of RD-114 RNA indicates a molecular weight of 5.0×10^6. Thus, if single subunits of RSV, FeLV, and RD-114 RNA contain all of the genomic information, there is about 60% more information content in RD-114 than in the other two common viruses.

The large subunit of RD-114 RNA has a rather unusual characteristic. In the otherwise smooth and well extended molecules observed in urea-formamide spreading there is a characteristic T or Y shaped secondary structure feature close to the middle of the molecule. This feature can be caused to denature so that RD-114 appears as a single smooth filament of molecular length 5.0×10^6 daltons, after extensive treatment with glyoxal.

Significance to Biomedical Research and the Program of the Institute: These studies represent a unique approach to cell transformation and cancer

induction by viruses. These studies could provide important information on the mechanism of viral transformation of cells resulting in cancer. In addition, it could lead to a new means of detecting the presence of the cancer virus genome in cells and could influence approaches to cancer prevention and/or therapy of the cellular level.

Proposed Course: 1) Extend the studies described above of the RD-114 RNA with corresponding studies of the RNA from CCC virus which is a closely related feline virus. 2) Study the RNA of baboon virus by electron microscopy since these viruses contain about 10 or 15 percent of RNA sequences in common with RD-114 virus. 3) In order to pursue the study of the sequence organization of integrated viral genomes methods are being developed for using ^{125}I labeled RNA for assaying the amount of tumor virus RNA sequences present in different DNA preparations. Studies will be initiated on fractionation procedures for the DNA of RD-114 cells in order to enrich for viral genomes.

Date Contract Initiated: March 1, 1974

Current Contract Level: $92,181

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (NO1-CP4-3209)

Title: Studies on the Possible Role of Oncogenic Viruses in the Causation of Cancer in Man

Contractor's Project Directors: Dr. Edwin H. Lennette
Dr. John Riggs

Project Officer (NCI): Dr. Paul Arnstein

Objectives: To apply the newer knowledge of the nature of RNA viruses to the study of neoplasms of animals and man.

Major Findings: Cell cultures derived from human laryngeal papillomas and genital warts have been co-cultivated with cells from different species in addition to human cell lines. In addition the cell cultures were treated with IDU and examined by electron microscopy (EM) for the presence of a papova virus. To date none of these approaches have shown promising results.

A hamster cell line initiated in this laboratory was shown by EM to contain three different viruses, a hamster type C virus, a hamster type A virus, and the hamster H virus. Studies are continuing in the isolation, purification, and characterization of especially the type A and type C viruses.

Serum produced in goats against murine leukemia virus proteins designated p30 and gp69-71 were obtained from Dr. J.T. August and were tested in the indirect fluorescent antibody procedure. The substrate cell infected with different mammalian type C viruses used in the test system was the D-17 dog cell line initiated in this laboratory. Both fixed and unfixed (membrane)

preparations were reacted and although p30 could not be detected at the cell membrane of the unfixed cells, it was evident that a great many cross reactions exist with both p30 and gp69-71 sera when tested by the IFA procedure. The anti MuLV antiserum reacted with type C viruses derived from the mouse, cat, and woolly monkey.

Pilot anti-tumor immunizations to NIH Swiss mice were performed with:

a) Live and killed murine cell lines (either virus-free or chronically infected with non-pathogenic type C virus).
b) Control vaccines FBS and Corynebacteria. Subsequent challenges using fibrosarcoma transplants or chemical carcinogen 3MC revealed the following (preliminary):

1. Live NIH Swiss cell lines, either virus free or infected with type C virus, give significant protection against fibrosarcoma transplant.
2. Killed infected cell lines apparently increase tumorigenicity of fibrosarcoma transplants. (Killed virus-free cellular vaccines do not).
3. Killed Corynebacterial vaccine gives significant protection against fibrosarcoma transplants, but FBS does not.
4. Chemical 3MC tumorigenicity is delayed and reduced in incidence by prior immunization with live type C virus infected cell vaccine and by Corynebacterial vaccine. 3M carcinogenesis was accelerated by preimmunization with killed cells containing the type C virus.
5. Indirect evidence suggests that the protection of non-tumored mice was cell (lymphocyte) mediated, rather than humoral antibody dependent.

X-irradiation: Techniques developed by H. Kaplan (Stanford) were repeated here, on C57/Leaden mice resulting in successful lymphoma induction in this mouse strain. Tumorigenicity of x-rays will be used in future vaccine studies.

Nude athymic mouse experiments: Nude mice have been used to propagate select human tumors. The use of currently available strain of "nudes" (on NIH Sw. x BALB/c stock) usually results in introduction of endogenous type C viruses; 3 types have been recovered (N-tropic, B-tropic and xenotropic). A less virus-prone "nude" is being developed by backcrosses to C57/Leaden mice which contain only low levels of xenotropic type C virus.

Ferritin-labeled antibody studies: Ferritin labeled antibody studies of xenotropic and ecotropic murine viruses revealed common virion envelope antigens among the xenotropic NZB virus, xenotropic NIH Swiss AT124 virus, xenotropic C57/L virus, Moloney and Rauscher leukemia viruses.

Significance to Biomedical Research and the Program of the Institute: The immunosuppression of mice with ATS and their subsequent tolerant state presents unique opportunities as an experimental system not afforded in

other methods of studying human cancers. One obvious advantage is the availability of a solid tumor, in which the human neoplastic tumor cells comprise the entire "malignant" component without the involvement of human subjects. These neoplasms are produced in the mice in 10-30 days and resemble architecturally well-known clinical tumor types. Thus, experiments can be designed to test chemical, physical or biologic influences on human solid tumors without the involvement of human subjects. The solid tumors might respond in a manner more closely resembling spontaneous human neoplasms than tumor cells in the more restrictive in vitro culture environment.

Proposed Course: 1) Growth of human tumor cells in ATS-treated mice for the purpose of a) verifying that they are tumor cell lines instead of the usual fibroblasts and b) trying to "switch-on" human type C virus by in vivo passage; 2) utilization of the ATS-treated mouse system for the study of viral vaccines; 3) utilization of the fluorescent antibody (FA) procedure to look for gs-1 antigens in human tumors and tumor cell cultures and also utilization of the indirect FA procedure to look for antibodies in human cancer patients or laboratory personnel working with candidate human type C viruses; 4) continue studies with human laryngeal papillomas to determine the viral involvement of papova viruses in the causation of these premalignant tumors.

Date Contract Initiated June 24, 1968

Current Contract Level: $245,818

CALIFORNIA, UNIVERSITY OF (NO1-CP3-3293)

Title: Studies on the Role of Virion-Associated DNA Polymerases in Malignant Transformation by Tumor Viruses

Contractor's Project Director: Dr. J. Michael Bishop

Project Officer (NCI): Dr. Edward M. Scolnick

Objectives: Conduct investigation on the molecular biology of the avian and other RNA tumor viruses, particularly a) the virion-associated DNA polymerase and b) the virus-specific DNA and RNA in normal and transformed cells.

Major Findings: The 4S primer on the genome of avian sarcoma viruses has the anticodon of tryptophan tRNA but can be aminoacylated with methionine. By contrast, an ostensibly identical RNA isolated from uninfected cells has all the properties of tryptophan tRNA. These results raise the possibility that association of primer with viral genome is either preceded or accompanied by a chemical modification which alters the interaction between primer and tRNA synthetases. Primer is bound by hydrogen bonds to a limited number of specific sites on the viral genome; none of the other 4S RNAs included in virions can bind to these sites. Purified primer can be annealed to high molecular weight subunits of ASV genomes, thereby forming a template-primer active with reverse transcriptase. Initiation of DNA synthesis on this

reconstructed template-primer and on native viral genome utilizes the same initial nucleotide sequence for template.

The production of mouse mammary tumor virus (MMTV) has been measured by a series of primary and long term cell cultures which provide courses of three major strains of the virus (MMTV-S, MMTV-P and MMTV-O). Virus production and the concentration of virus-specific RNA in one line of cells (GR) is increased ten- to twenty-fold by the presence of dexamethasone in the medium. Optimal doses of the hormone and the kinetics of the response to the hormone have been determined. The response is not affected by inhibition of cellular protein synthesis. This system is a promising tool for the study of the mechanisms which mediate phenotypic responses to corticosteroids. Consequently, a collaborative study has been initiated with Dr. Gordon Tomkins.

The genomes of MMTV endogenous to various strains of mice are being compared by molecular hybridization. Continued refinement of this approach has now led to indications that the DNA from different strains of mice may contain different amounts of MMTV-specific nucleotide sequences, and that these sequences have diverged somewhat in the different mice. The use of a restriction endonuclease has provided preliminary evidence that the MMTV proviruses in BALB/c and RIII mice are adjoined by different sets of nucleotide sequences, or that the gene sequences of the MMTV harbored by these mice are different in the two strains. Proviral DNA for MMTV appears to occur only in the genus Mus (including feral Mus Musculus and Asiatic mice).

Analysis of RNA and DNA from carcinomas of the human breast continues. RNA from two of forty tumors demonstrated appreciable homology with the genome of MMTV. By contrast, no homology between human DNA and MMTV has been detected.

Significance to Biomedical Research and the Program of the Institute: These studies are providing an important insight into the mechanism by which RNA tumor viruses bring about malignant transformation, and perhaps will lead to significant advances in the understanding of the causation and control of human neoplastic disease.

Proposed Course: 1) The nature of 4S RNA primer on 70S RNA. Studies of the nucleotide composition and sequence will be continued, along with efforts to characterize and locate the binding sites for primer on 70S RNA. 2) Determine the nature of the transformation information contained in Rous sarcoma virus. 3) Mouse mammary tumor viruses. a) Use molecular hybridization to compare the MMTV genes present in various strains of mice and to analyze the extent of relatedness among the several variants of the virus. b) Use molecular hybridization to search for biologically inapparent infection of rodents other than mice. Attempt to infect the germ line of mice and other rodents with GR virus, resulting in the establishment of genetic transmission of the viral genome. These infections may be biologically inapparent. If established, they can be used to dissect the nature of the controls which limit the expression of MMTV genes in genetically infected mice. c) Analyze

the distribution of integration sites for MMTV genomes in the DNA of different mouse strains in order to ascertain whether the site of integration is a determinant of gene expression. d) Molecular hybridization with human materials. Further efforts will be made to detect hybridization between MMTV-specific nucleic acids and both RNA and DNA from various human tissues and attempt to characterize the putative type B particles found in human milk. e) Investigate the role of corticosteroids in the production of MMTV by cultured cells.

Date Contract Initiated: June 2, 1971

Current Contract Level: $220,000

CALIFORNIA, UNIVERSITY OF (NO1-CP3-3242)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Thomas Kawakami

Project Officer (NCI): Dr. Wade P. Parks

Objectives: To continue studies of the in vitro characterization of the woolly, gibbon and other simian type C viruses, especially by antigenic and biochemical characterization; to continue studies of the pathogenicity of the simian viruses; to evaluate the natural history of the gibbon type C virus in specimens from Thailand and San Francisco Zoo populations; to employ serologic studies to detect shared antigenic components between the simian viruses and putative human type C viruses; to isolate or identify viruses or viral antigens in spontaneous human tumors as well as in nonhuman primate tumors using a wide variety of in vitro techniques.

Major Findings: Virus detection, isolation and characterization from nonhuman primates and human tissue continued to be emphasized utilizing established in vitro techniques. Since September two nonhuman primate and 24 human tumors were or are being evaluated for type C virus by electron microscopy, DNA polymerase assay, ^{3}H-uridine incorporation and serologic tests. Additional rhesus monkey tissues examined for type C viruses proved to be negative; however, seven additional gibbon type C viruses were isolated from peripheral blood cells of clinically normal gibbons. The viruses isolated from the gibbons were found to share antigenic determinants on the envelope, 30,000 dalton virus protein, and viral-DNA polymerase with known primate type C virus. The relatedness of the virus was established by IFA, neutralization, immunodiffusion and polymerase immunoinhibition tests. Biochemical studies on the primate type C viruses were continued to study the conditions for optimal activity and differentiation of the viral and host cell enzymes. Viral polymerases from different animal viruses showed similar preference for synthetic templates under identical conditions with the exception of the avian myeloblastosis virus. Avian virus differed in showing a much higher utilization of oligo dG-poly rC compared to simian and feline viruses.

Examination of human tumors and placentas by electron microscopy, culture initiation and co-cultivation, and other viral activation and detection methods has yielded presumptive evidence of RNA virus in only a few instances. Human tumor with evidence of virus-like particles lacked DNA polymerase and showed no evidence of being infectious to other cell cultures. Human placenta with evidence of type C virus by electron microscopy showed low DNA polymerase activity in tissue culture fluid, but the cells could not be propagated. Placenta cells co-cultivated with heterospecies cell cultures showed increased polymerase activity but the infection could not be maintained.

Studies on gibbon and woolly monkey immune response to type C virus after infection with the homologous primate virus showed that these animals developed antibody to both the envelope and group specific viral antigen. Antibodies to the envelope antigen of the primate type C virus in gibbons and woolly monkey sera were detected by indirect immunofluorescence and radio-immune assay, while antibodies to the group specific antigen were detected by complement fixation and immunodiffusion tests. Gibbons viremic with type C virus were found to lack antibody reactive to the virus. All gibbons are being monitored for clinical changes and for type C virus antigen and antibody.

Significance to Biomedical Research and the Program of the Institute: The finding at this laboratory of two type C viruses associated with tumors of different primate species is evidence that the higher animals, including man, are likely to be among the growing number of species harboring type C viruses. Since monkeys and man are closely related phylogenetically, the proposed studies, which are oriented toward characterization of the primate viruses and seeking possible relation with human tumors, are of direct relevance in establishing the etiology of human cancer.

Proposed Course: 1) Efforts will continue to detect, isolate and characterize viral agents from spontaneous nonhuman primate and human tumors, utilizing various rescue or viral activation methods. 2) Characterization of recently isolated type C primate viruses and human virus-like particles in tumors and placentas will be continued. Comparison studies with known type C viruses will be included. 3) Epidemiologic studies on primate viruses for evidence of infection in human and animal species associated with gibbons will be continued. 4) Further pathogenesis studies will be continued to establish the oncogenicity of SLV, SMV, and other type C primate viruses. These studies will involve monitoring the animals for clinical changes, including immune response to type C viruses.

Date Contract Initiated: November 1, 1969

Current Contract Level: $497,664

CALIFORNIA, UNIVERSITY OF (NO1-CP4-3381)

Title: Isolation of Human Xenotropic Viruses

Contractor's Project Director: Dr. Jay A. Levy

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Conduct investigation on the isolation of a human type C virus using techniques developed in demonstrating type C viruses in other animal systems, particularly the xenotropic virus first detected in New Zealand Black mice. Specific emphasis will be placed on the cultivation and study of tumors from patients with autoimmune disease and malignancies associated with immunologic disorders.

Major Findings: Placentas from four patients with systemic lupus erythematosus (SLE) and kidney biopsy tissues from two SLE patients and a fibroma from one SLE patient were cultured. Tissue minces from the placentas were cultivated with cell lines of rat, mouse, rabbit and human origin.

Sections from the trophoblast, maternal and fetal portions of four placentas were prepared for electron microscopy by routine methods. In the maternal layer of two of these placentas type C particles were present. Examination of the last two placentas obtained is still in progress.

From each of the four placentas placed in culture, ten cell lines have been established and tested for the presence of biologically active type C viruses. One cell line cultivated from the second placenta has, on one occasion, shown low levels of reverse transcriptase activity but no type C particles have been observed in these cells by electron microscopy.

Sera from lupus patients have been tested extensively for antibodies to mouse xenotropic and ecotropic viruses as well as rat ecotropic virus. No reactivity has been detected. Sera have also been examined for inhibitory activity of type C virus infection in tissue culture and no significant effort has been noted.

The establishment of lymphoblastic cell lines from a patient with T cell leukemia and another with chronic lymphocytic leukemia has also been attempted. After four months in culture, no continuous lines have resulted, but viable lymphocytes are still present in those cultures containing human foreskin cell feeder monolayers.

Significance to Biomedical Research and the Program of the Institute: It has become apparent, based on work on the mouse system, that type C viruses do exist which can be produced actively by the host but cannot be propagated in other host cells. This observation may well explain the failure thus far at isolating a human type C virus in pure culture. Once accomplished, this human virus will be most important in looking not only at its pathology in other hosts but also as an immunologic tool for detecting other potential human type C viruses in human tumors and tissues. Moreover, only after

cultivation in vitro can a virus be available for studying its possible role in normal cellular physiology as well as human malignancy.

Proposed Course: Continue to search for the presence of type C xenotropic virus in human tissues.

Date Contract Initiated: June 27, 1974

Current Contract Level: $101,049

DEPARTMENT OF AGRICULTURE (BELTSVILLE) (YO1-CP-4-0214)

Title: Inheritance and Oncogenicity of RNA Tumor Viruses

Contractor's Project Director: Dr. L. B. Crittendon

Project Officer (NCI): Dr. Padman Sarma

Objectives: These studies will make use of inbred chicken lines to define the genes involved in controlling expression of the infectious endogenous type C RNA chicken viruses (RAV-0) and will determine whether the RAV-0 virus is oncogenic.

Major Findings: Preliminary studies at the USDA aimed at defining the genetic control of resistance to and expression of endogenous avian type C viruses (RAV-0) have resulted in lines which consistently produce RAV-0 and others which do not, and that RAV-0 production is independent of genetic resistance to the virus. To date the endogenous virus does not appear to contribute to chicken neoplasia, at least during the commercial lifetime of the chicken. However, commercial flocks are kept for a period of months which represents little beyond the infancy of a relatively long-lived bird. This finding would be consistent with observations of spontaneously occurring cancer in other species, in which cancer is a disease of old age.

Recent studies have shown that two RAV-0 producing chicken lines were crossed with a low virus producing susceptible line. Nearly all embryos produced RAV-0 suggesting that dominant genes control spontaneous RAV-0 production. Segregation of backcrosses to the low producing line female suggested single dominant gene inheritance. Further matings are in progress to test this hypothesis. Embryos from backcrosses using the viremic F_1 dams gave a very high incidence of RAV-0 production suggesting congenital maternal transmission.

An experiment comparing the incidence of lymphoid leukosis after spontaneous and artificial exposure to RAV-0 with artificial exposure to RAV-1 (a typical lymphoid leukosis virus of subgroup A) showed that RAV-0 was very inefficient at inducing lymphoid leukosis compared to RAV-1. Therefore, RAV-0 either has no oncogenic potential or is poorly oncogenic. The epidemiology of RAV-0 infection in susceptible non-virus-producing chickens resembled that of RAV-1, an exogenous virus.

The expression of RAV-O in two types of embryos which produce at a very low rate was studied. Cells from RAV-O producing resistant embryos appeared to have the potential to release complete RAV-O, but this happened only occasionally. Cells from our line of group-specific (gs) antigen positive chickens appeared to produce RAV-O at an extremely low rate in contrast with other lines which express gs antigen, but no virus is detectable. Further studies on the potential for RAV-O production in these cell types are in progress.

Significance to Biomedical Research and the Program of the Institute: It is increasingly apparent that many vertebrates, including primates, carry information for complete RNA tumor virus expression, including oncogenic potential, in their genomes. The endogenous origin of this viral information changes our concepts of the control of at least some types of cancer from methods which eliminate or control exogenous viruses to mechanisms which suppress endogenous viral information. These studies provide another system, in addition to the mouse, where the genes controlling production of the endogenous type C virus will be defined. They will provide another system for defining the role of the endogenous virus in naturally occurring neoplasms.

Proposed Course: Continue to study the influence of endogenous and exogenous RAV-O and the presence of natural gs antigen on the incidence of lymphoid leukosis in adult chickens.

Date Contract Initiated: July 1, 1974

Current Contract Level: $30,000

FLOW LABORATORIES, INC. (NO1-CP5-3530)

Title: Studies of Type C Viruses in Relation to Oncogenic Potential

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct immunological and serological studies of oncogenic type C RNA viruses associated with neoplasia. To develop immunologic, biologic, and biochemical reagents and techniques for identification of type C viruses and their gene products.

Major Findings: Inter- and intra-species relationships among the major internal proteins (p30) of mammalian type C viruses were studied by immunochemical, biophysical, and structural techniques. p30s of a variety of MuLV strains appear to be >95% related based on quantitative micro-complement fixation, the few percent differences being reflected in minimal but distinct differences in tryptic peptide maps. NH_2-terminal sequence analysis showed only one difference of 25 residues compared among MuLV p30s, strikingly three different residues have been found at residue 4, the only polymorphic residue found thus far. Microheterogeneity in isoelectric point, probably

indicating post-translational modifications, have been found for all MuLV p30s. Enzymatic cleavage and chemical modification experiments have shown that p30s are antigenic mosaics with multiple species and interspecies determinants. The definition of these determinants is being accomplished by isolation of reactive peptides, sequencing and synthesis; the latter in collaboration with Dr. Stephen Sallay, Purdue University. In terms of interspecies relationships, the striking finding has been the very close relationship of baboon and RD-114 p30s giving identity reaction in gel diffusion and allowing use of reciprocal reagents in in vitro tissue culture assays (the latter in collaboration with Dr. Robert McAllister, Los Angeles). This relationship extends to other virion proteins and assays including the KC syncytial assay. Very recently it was found that NH_2-terminal 14 residues of baboon and RD-114 p30s were identical. Based on this relationship and findings of others (Sherr, Benveniste, Todaro) extensive attempts to detect p30 determinants using a variety of direct and indirect immunoassays in human tumors are underway, thus far without definitive results. Included also in these studies are tests for interspecies determinants using a reagent prepared by sequential immunization with various p30s. This results in an immune serum qualitatively and quantitatively enriched in interspecies reactive antibody. Studies with other virion proteins, p10, p15, and gp70 are proceeding with the development of purification methods and specific antisera. p15 (or one of several components in this size range) appears to be a surface component which because of involvement in cytotoxic reactions (the FMR antigen appears to be p15 - Lilly) may be important for subunit vaccine purposes. Both species and type-specific determinants associated with p15 have been identified in gel diffusion studies. A possible precursor to the low molecular weight polypeptides has been found in mouse L-cells. A 70,000 M.W. protein was isolated which reacted with sera specific for p30 and p15. Studies of the polypeptides from a single virus type have shown unique determinants associated with each.

A variety of human sera including those from cancer patients, individuals with connective tissue disorders, and laboratory workers were tested for p30 interspecies antibody in RIA with negative results. A survey of human sera for antibody to the "bovine leukemia virus" obtained from Van der Maaten and Miller (Ames, Iowa) also gave negative results. This virus has a Mg^{++} dependent RNA dependent DNA polymerase (like "B-type" viruses) and a major internal protein of 24,000 M.W. which lacks type C interspecies antigenic determinants.

A survey of hamster tissues for HaLV p30 showed widespread presence of antigen in embryonic and adult tissue at levels below that required for reaction in CF tests. These results were verified by concentration of reactive antigen on immunoaffinity columns.

Molecular hybridization studies have shown a high degree of species specificity for mouse, hamster, and rat endogenous viruses. This has made possible the detection of nucleic sequences corresponding to the species of origin in interspecies pseudotype viruses. One case of extreme interest is the B-34 virus (R. Bassin, NCI) which was isolated from hamster tumors induced by Harvey-MSV. B-34 contains only hamster viral proteins but has RNA sequences of mouse, rat, and hamster. These remain unchanged from the parental viruses based on thermal stability experiments. B-34 complementary DNA (cDNA)

prepared in endogenous reactions in vitro is mainly mouse specific, indicating asymmetric reverse transcription. Thus, one sees the obvious problem of lack of agreement between immunological and hybridization results which are clear only when the virus history is known. Also, it was found that a HaLV strain (termed GLOH-) isolated by endpoint dilution from a sarcomagenic virus isolated from hamster tumors induced by the Gross pseudotype of M-MSV contains sequences corresponding to hamster and mouse viruses. Based on thermal stability these mouse sequences are M-MSV related and distinct from GLV. Authentic hamster virus nucleic acid sequences were found in hamster cell DNA and rat viral sequences in rat cell DNA.

The gibbon virus was shown to cross-hybridize to endogenous hamster viruses in addition to the previously reported results with mouse viruses. One wonders, based on immunologic uniqueness, whether this and the woolly monkey virus are not examples of viruses which have undergone reassortment under natural conditions.

Sequences related to the Kirsten sarcoma virus were found in several non-transforming rat type C viruses and in normal rat cells in vitro. This RNA species sediments at 30S and is distinguishable from the 35S RNA also found in rat viruses and infected cells. 35S RNA was not found in normal rat cells. The 30S KiSV specific RNA was found in mouse nonproducer cells transformed by KiSV including variants induced to produce virus by BrdU. The specific probe was prepared by absorbing cDNA prepared from one such activated virus with mouse viral RNA. The KiSV specific cDNA does not anneal with normal mouse cell RNA nor with any mouse type C virus tested. It was reported previously that M-MSV sequences in nonproducer cells and in virion populations (after heat or DMSO treatment) with excess transforming ability also sedimented at 30S. The KiSV and M-MSV sequences do not show relationship by hybridization, but we would predict some functional relationship.

Attempts to show nucleic acid sequences in either RNA or DNA corresponding to known type C viruses in human tumor cell lines including those with EB genomes have been negative to date.

cDNA intermediates in viral replication have been found in unintegrated form within 40-70 minutes after infection. Both low molecular weight fragments and molecules corresponding to expected genome size have been detected by hybridization techniques Both negative (initial copy) and positive strands have been detected. The results are consistent with the conversion of input labeled viral RNA to a density characteristic of RNA-DNA hybrids in the same time period (in collaboration with T. Takano). These intermediates should prove useful for hybridization, biological, and structural studies.

The KC syncytial assay was found applicable to titration of Mason-Pfizer virus providing a plaque assay. The results of this assay correlated with production of MP-MV p27 and Mg^{++} dependent polymerase activity. Thus, this assay detects RD-114 and baboon virus among the type C viruses and MP-MV among the B types. All other reverse transcriptase containing viruses have been found negative. We have used this assay in conjunction with RIA and polymerase assays in attempts to detect viral activity in human

cell lines. Fifty cell strains established from placentas and a number of human tumor cell lines (obtained from Drs. R. M. McAllister and W. P. Parks) were tested directly, after IudR treatment, and after co-cultivation with primate, dog, mink, bat, rat, and mouse cells. Thus far no evidence of viral activity has been found. Two cell lines derived from placentas and producing chorionic gonadotropins were found to give syncytia in the KC test. These lines are being explored in detail, thus far with no evidence of viral antigens.

A soluble inhibitor of fusion in the KC assay was isolated from RD-114 virus. This appears to be an isolated envelope glycoprotein.

Standard pools of helper and sarcoma viruses were prepared for use in vaccine-related studies. Neutralizing antibody was prepared in goats to several MuLV strains including Rauscher, RadLV, and AT 124 (xenotropic virus). These are to be used in passive immunity studies. Routine testing of mouse sera and human sera against mouse and other viruses is in progress. These tests are to evaluate the efficacy of formalinized vaccines prepared under NO1-CP-43387.

Significance to Biomedical Research and the Program of the Institute: The contract is a major source of reagents for typing type C tumor viruses. The expertise developed by the contract for purifying viruses and subviral components and for preparing antisera of known specificity has allowed rapid and reliable identification of viruses isolated from various species. These reagents are especially important in instances where virus isolated have been obtained from human tissues. Since the human type C virus has yet to be isolated and characterized, it is important to have reagents available for testing isolates to exclude contamination by nonhuman viruses. The contractor is also involved in biological and biomedical studies relating to the mechanism of type C virus persistence in cells and the differentiation of events leading to cell transformation. The existing expertise of the contractor and the programs presently under development will prove useful in attempts to develop vaccines for immunoprevention and immunotherapy of human tumors.

Proposed Course: Continuation of studies summarized above with emphasis on development of highly specific, well-characterized reagents, both of immunological and nucleic acid type. Continuation of attempts to define and control the transformed state; e.g., DNA binding proteins, transport sites. Performance of surveys utilizing immunological and molecular biological techniques on relevant materials.

Date Contract Initiated: February 1, 1971

Current Contract Level: $2,000,000

HARVARD UNIVERSITY (NO1-CP3-3265)

Title: Primary Structure and Synthesis of Avian Leukosis Virus Proteins

Contractor's Project Director: Dr. Geoffrey Tregear

Project Officer (NCI): Dr. Padman Sarma

Objectives: 1) To complete the determination of the primary structure of the gs-a and gs-b antigens of avian myeloblastosis virus (AMV), and 2) to isolate and sequence additional group-specific (gs) antigens from AMV.

Major Findings: In a previous summary report, the synthesis of the wild mouse leukemia virus (WMLV) peptides corresponding to the sequence 1-12 and 4-10 was described. The optimal experimental condition developed for the synthesis of these peptides has now been applied to the synthesis of two further peptides of the wild mouse sequence. Peptides representing the WMLV amino-terminal sequence 1-24 and 12-24 have been successfully synthesized, purified and evaluated for chemical purity.

Quantities of the synthetic peptides have been made available to Dr. Raymond Gilden of Flow Laboratories Inc. for use in the development of specific radioimmunoassay procedures for the detection and assay of leukemia virus group-specific antigens.

Significance to Biomedical Research and the Program of the Institute: The determination of the primary structure of the gs-a and gs-b antigens of AMV are a prerequisite to the chemical synthesis of one (gs-b) or both of these antigens. The avian myeloblastosis virus is being used as a model system prior to initiating similar studies with the interspecies antigen (gs-3) of mammalian viruses. The availablility of a highly specific and purified antibody against the interspecies antigen would considerably aid in finding antigenic fingerprints of a potentially oncogenic virus in human neoplastic tissue.

The contract was terminated on December 31, 1974.

Date Contract Initiated: September 18, 1972

Current Contract Level: No FY75 funds

JACKSON LABORATORY (NO1-CP3-3255)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host Gene Control of Their Expressions

Contractor's Project Director: Dr. Hans Meier
Dr. Hans-Jorg Heiniger
Dr. Benjamin Taylor

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and resistance to cancer and the RNA tumor viruses. The Jackson Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on type C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings:

1. Genetic programming for primary tumors and expression of murine leukemia virus. The expression of endogenous type C RNA tumor virus was found to be under the control of two genes in AKR x C57L crosses and the occurrence of tumors was predictable according to the genotype. In addition, we found that other genes, such as Mlv-1, hr, AY, and dw, affect virus replication and/or tumor incidences in addition to Fv-1 and Fv-2.

2. Relationship of viral expression and tumor development with various host marker genes. Apart from the strong correlation between the expression of gs-1 antigen and tumor incidence attributable to the genes Akv-1 and Akv-2 in the AKR x C57L crosses, we also found a weak relationship between the H-2 genotype and leukemia development. A possible relationship of dipeptidase-1 (Dip-1) locus and tumor incidence as well as esterase-1 (Es-1) is currently being analyzed further. Also, linkage of the loci ln, Dip-1, and ald has been established.

3. Genetic and biochemical studies of malignant and blastogenic transformation. By use of our recently developed micromethod for the culture of peripheral mouse lymphocytes, we analyzed the heritability of the transformation process; we estimate that 2 to 4 genes are involved. We further determined that leukemic lymphocytes have a 300-fold increased cholesterol synthesis in their membranes compared to normal lymphocytes; there is no noticeable increase of cholesterol levels in the leukemic serum. The significance of these findings is presently pursued in an effort to better understand the mechanism of malignant transformation and the interaction of lymphoid cells with the type C tumor viruses.

4. Viral-chemical and -physical cocarcinogenesis and testing of viral vaccines. We have exploited several systems inducing complete type C RNA virus and tumors with chemical and physical agents. In virus negative SWR-cells we induced the production of complete virus with a single dose of dimethylnitrosamine (DMN). The virus, however, is non-infectious and replication-deficient. In the congenic strains, B10.D2/n and B10.D2/o, leukemias were induced after repeated x-ray exposure and significant differences in the occurrence of the malignancy were observed. It is postulated that immune reactions dependent upon the presence of complete complement (lacking in B10.D2/o mice) may be deficient and lead to an

accelerated tumor growth. Various mouse strains have been exposed to single and multiple doses of several carcinogens (DEN, MCA, DMN, ENU). It was found that the AKR x SWR cross and its reciprocal are unique tools for inducing high and early incidences of leukemias and pulmonary adenomas. This model is presently used to test the effect of a variety of viral vaccines on tumor incidence.

5. Genetic studies of type B (mammary) tumor virus expression. We analyzed the amount of MMTV gp-52 in milk from different inbred strains of mice. The titers vary among strains and are influenced by parity. Second parity milk yielded most reliable results and is now used for further genetic studies in recombinant inbred lines (RI) originating from crosses of C3H/HeJ x C57BL/6J mice. By this approach we aim to map the genes controlling the expression of gp-52 as several of the BXH lines not only differ markedly from one another in gp-52 expression but also in various marker genes for which they were typed.

Significance to Biomedical Research and the Program of the Institute: This program has contributed much of the basic data concerning the genetic determinants of oncogenesis and the natural expressions of the endogenous type C virus. It has pointed up the overwhelming influence of genetic predisposition in the development of natural cancer and susceptibility to environmental carcinogens. The contractor has developed sophisticated systems for defining and locating the genes and loci involved in murine oncogenesis, and virus and antigen expression, and has rescued complete virus through gene complementation by hybridization of two virus-free mouse strains.

Proposed Course: 1) Further development, characterization, and uses of recombinant inbred lines. 2) Study of genetic control of endogenous murine leukemia virus. 3) Study of association of the viral group-specific antigen with tumor development. 4) Genetic analysis of a dominant gene inhibiting expression of the group-specific antigen. 5) Mapping of viral structural genes by use of defective sarcoma virus mutants: complementation classes. 6) Additional marker studies for mapping structural genes of viral components. 7) Induction of complete or partial virus synthesis by drugs. 8) Chemical co-carcinogenesis studies. 9) Marker studies (immunogenetic and biochemical). 10) Study of genetic control of embryonic and post-natal, normal and abnormal cell proliferation. 11) Study of the effect(s) of hormonal stimulation of RNA metabolism in dwarf mice. 12) Host genetic rescue of murine leukemia virus.

Date Contract Initiated: May 2, 1967

Current Contract Level: $441,246

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP3-3248)

Title: Immunoprevention of Spontaneously Occurring Neoplasms

Contractor's Project Director: Dr. Robert M. Nims

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: The primary objective is to study immunoprevention of spontaneously occurring tumors in laboratory mice by the use of viral and cellular vaccines. The contractor has extensively studied the parameters involved in spontaneous neoplasia, including the incidence, progression and histological types of neoplasms in several strains of mice. Additionally, the relationship of the occurrence of the neoplastic state to the host's indigenous type C virus expression has been well defined. The important parameter remaining, the host's cellular and humoral immune response to its neoplasms and type C virus, has been under study in the past contract year. The additional methodologies required to pursue these objectives have been developed. These include a footpad assay for measurement of delayed type hypersensitivity in the mouse, techniques for lymphocyte culture and the development of a lymphoblast transformation assay for type C viruses and tumor cells.

Major Findings: This contract's primary objective is to examine the possibility of immunoprevention of spontaneous neoplasms in animal model systems that have viral and epidemiologic similarities to human neoplasia. Development of assays for cell-mediated immunity to type C viruses are a necessary prerequisite for these studies and have received major emphasis this past contract year.

An *in vivo* assay for delayed type hypersensitivity (footpad test) was developed to assess the induction of cellular immunity to murine leukemia virus (MLV) antigens. The response was best induced by the subcutaneous injection of antigen in adjuvant (Freund's Complete). Formalinized, ether-extracted immunogen (Rauscher, AKR- and Gross-MLV) showed type specificity when sensitized mice were challenged with ether-extracted MLV. Mice immunized with formalinized, ether-extracted R-MLV resisted challenge with 100 x the 50% infectious dose of R-MLV, and also, to 10 x 50% infectious dose of the naturally occurring BALB/c leukemia virus.

In vitro tests for cellular immunity to MLV and tumor cell antigens have been developed, including cell-mediated cytotoxicity and a micro-adapted version of the lymphocyte transformation assay. *In vivo* schedules, dosages and routes for immunization as well as *in vitro* challenge procedures have been evaluated for both assays. Immunity to a syngeneic spontaneous tumor cell line was effected by use of an x-irradiated cell vaccine and demonstrated by lymphocyte mediated cytotoxicity. Specific and cross reactive stimulation of lymphocytes by MLV antigens have been demonstrated in immunized mice.

This laboratory also performed *in vivo* testing for cell tumorigenicity utilizing over 600 rats and prepared and evaluated 15,000 histologic slides for VCP and collaborating investigators.

Significance to Biomedical Research and the Program of the Institute: This contract has contributed significantly to 1) experimental findings from which the oncogene hypothesis was formulated, 2) demonstrating the prevalence of the type C viral genome during normal embryogenesis, and 3) to an understanding of the endogenous type C virus relationship with its host and its genetic and epigenetic relationship to the host's naturally occurring neoplasm. The current studies on immunoprevention of spontaneously occurring tumors are designed to answer the question of whether neoplastic disease can be prevented or treated by classical virological vaccine or cellular vaccine techniques.

Proposed Course: 1) Completion of studies designed to develop usable techniques for monitoring host cellular immunity including the footpad assay, the lymphoblast transformation assay, and a cell-mediated and humoral cytotoxicity assay. 2) Immunoprevention studies with viral vaccines will include challenge with exogenous leukemia viruses, challenge with tumor cell and tumor transplant lines, and holding animals for prevention of spontaneous tumors. 3) Immunoprevention studies with cellular vaccines will include challenge with exogenous leukemia viruses, challenge with tumor cell and tumor transplant lines and holding animals for prevention of spontaneous tumors. The importance of the immunogenicity of type C virus in cellular vaccines will be examined.

Date Contract Initiated: November 15, 1961

Current Contract Level: $695,000

MICROBIOLOGICAL ASSOCIATES, INC. and CHILDREN'S HOSPITAL OF AKRON
(NO1-CP4-3240)

Title: The Roles of Viruses and Chemicals in the Etiology of Cancer and its Prevention

Contractor's Project Directors: Dr. Mina Lee Vernon
Dr. Paul Price
Dr. Ilan Shif
Dr. Richard E. Kouri
Dr. Aaron E. Freeman (Children's Hospital of Akron)
Dr. Howard Igel (Children's Hospital of Akron)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Primary emphasis is placed on the following major tasks: 1) Develop, evaluate, standardize and apply in vitro systems for studying the effects of known and suspected carcinogens in the environment. 2) Utilize in vitro systems and temperature-sensitive mutants of type C RNA murine viruses to elucidate viral and cellular components involved in cell transformation. 3) Utilize cell systems to study potential anti-cancer products, including viral vaccines, viral inhibitors, interferon, etc. on naturally

occurring and chemically induced tumors. 4) Utilize and adapt subhuman mammalian transformation and viral induction procedures to studies of human tumor and transformed cell lines for assaying suspected environmental carcinogens.

Major Findings: 1) The H43 clone of F1706 Fischer rat embryo cells was used to determine the transforming potential of food colorings in double-blind testing. Two green, 2 red and 1 blue dye transformed the cells and rendered them tumorigenic in newborn Fischer rats. The transforming potential of 10 dyes has been determined; 10 are on test and 10 will be on test in the near future. In two separate experiments, the drug Tofranil was found to be a weak transforming agent. 2) In preliminary studies, cordycepin and narcissus alkaloid A-3 protected rat embryo cells from transformation by 3MC. Cordycepin also inhibited IDU induction of endogenous rat virus by 30%. At non-toxic levels, sialic acid reduced the titer of free RLV by almost 3 logs; when removed the cells resume normal release of viruses into the medium. Adriamycin did not affect IDU virus induction or protect against 3MC transformation; rather, it was a transforming agent. 3) Fischer rat embryo cells chronically infected with Rauscher leukemia virus were protected from transformation with 3MC by pretreatment with RLV rabbit antiserum; pretreatment with control rabbit serum did not protect. Optimum conditions for transformation using temperature sensitive mutants of RLV are being studied. 4) Several transformation systems developed in this and other laboratories are being evaluated with the objective of having a battery of in vitro tests. The most promising 3 or 4 systems will be used in double-blind tests. 5) Fetal hamster cells transformed with ara-c can occur under conditions of little or no inhibition of DNA synthesis or cytotoxicity, require only a 6 hour exposure, seem to require DNA synthesis and transformed colonies produce fibrosarcomas in syngeneic newborns, but this system was not sufficiently sensitive or reproducible for screening using a blind protocol. 6) Only if the number of 5_7 chromosomes are greater than 7_3 chromosomes are ara-c transformed clones malignant in vivo. 7) Some ara-c transformed clones passed in vivo express virus with some characteristics of type C viruses (H^3-uridine uptake, banding at 1.16 70s RNA IDU induction, gs antigen and EM morphology). The virus will not grow in hamster, rat, mouse, human or rabbit cells, contains RT activity that will not read its own 70s RNA, reads poorly poly r(A)-oligo d(T) or poly r(C)-oligo d(G) and not at all the DNA template poly d(A)-oligo d(T). Mn^{++} and 0.33-1.0% TritonX-100 are required to detect RT activity. 8) Development of a human cell transformation system is being attempted using human cells chronically infected with mouse xenotropic type C virus. 9) Sera of C57BL/6 mice treated with 3MC were assayed for evidence of an effect on X-tropic virus antibody levels but no differences were seen. Similarly, no differences were detected in the expression of X-tropic or ecotropic viruses in tumors, spleens and kidneys of C57BL/6 mice treated with 3MC. Breeding experiments to study genetic regulation of X-tropic virus have been initiated. 10) Parallel studies using tissue culture interferon and mouse serum interferon showed no effect by TC or MS interferon on tumor incidence, average tumor latency or C1 values of CF-1 mice treated with 3MC as neonates. Sub contract: 1) Matched pairs of non-infected cultures and cultures infected with RaLV or RLV were carried 10 passages to test the slow transforming potential of type C RNA viruses. No evidence of transformation has been detected. 2) Over 30 human cell cultures were treated with

halogenated nucleotides and chemical carcinogens with no evidence of transformation so this approach was discontinued. 3) Foci of human epithelial cells obtained on killed pig skin were treated singly and in combination with urethane, 3MC, and BuDR, and are being observed for morphological change. No problems are anticipated that would prevent continuation of the studies being conducted under this contract.

Significance to Biomedical Research and the Program of the Institute: This contract is a core part of a coordinated, comprehensive, targeted research program to define the role of the type C RNA genome as determinant of oncogenesis, to develop diagnostic systems for screening carcinogens, and to continue studies with vaccines, interferon, and other potential candidates to inhibit cell transformation and/or prevent or control cancer. This program has contributed profoundly to the development of new approaches and concepts, which in turn have provided new insights into the nature of the oncogene, and mechanisms of viral and chemical carcinogenesis. Specifically, the in vitro tissue culture systems for assaying environmental carcinogens make possible large scale, rapid screening of environmental compounds, drugs, food additives, etc., at a fraction of the cost of equivalent tests in vivo. In view of the screening burden posed by literally thousands of new compounds each year, the newly developed tissue culture systems represent the only feasible hope of recognizing possibly dangerous carcinogens before the public has been harmed.

The development of viral vaccines capable of immunizing against chemical carcinogenesis, and optimistic results with mouse interferon against chemically induced tumors in animals, have important implications for cancer prevention and control in the near future. The inital tests have established that vaccines which confer greatest protection are those derived from "natural" type C viruses, leading to the conclusion that immunity is antigen-specific. These results are particularly encouraging with the advent of new methods for inducing human type C viruses. The finding of type C viruses in human placentas has added considerable impetus to the search for other human type C virus candidates.

Proposed Course: Work will be continued on 1) the development, evaluation and standardization of in vivo and in vitro systems for studying the effects of known and suspected environmental carcinogens; 2) development and application of sensitive in vitro assay systems for studying the natural history of type C tumor virus infection and its relationship to carcinogenesis in a variety of mammalian systems; 3) development and testing of type C virus vaccines, interferon and anti-transformation compounds to evaluate their efficacy in prevention of spontaneous and chemically-induced tumors in genetically defined mouse strains and cell systems.

Date Contract Initiated: February 1, 1970

Current Contract Level: $839,525

MICROBIOLOGICAL ASSOCIATES (NO1-CP5-3519)
(Formerly part of contract NO1-CP4-3240)

Title: Isolation and Characterization of Type C RNA-Tumor Viruses and Diagnostic Testing and Service Functions

Contractor's Project Directors: Dr. Mina Lee Vernon
Dr. Paul Price
Dr. Ilan Shif
Dr. K. Higuchi
Dr. John Parker
Dr. Richard Kouri
Dr. Johng Rhim

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: 1) Utilize established nonproducer lines in a variety of co-cultivation techniques and treated with chemicals in an effort to activate or enhance expression of endogenous viruses or viral genomes and in efforts to detect and "rescue" human type C RNA viruses or viral genomes. 2) Define the events, with special emphasis on the role of endogenous type C RNA viral genomes, associated with activation of viruses in tissue culture by chemicals, DNA viruses or spontaneously, and characterization of endogenous viral isolates. 3) Conduct natural history studies of the overt expressions as seen in EM of Types A, B and C RNA viruses in several species and evaluate in EM the effects of carcinogens, hormones, etc., on activation of detectable viral particles. 4) Study the role of the enzyme aryl hydrocarbon hydroxylase (AHH) in chemical carcinogenesis. 5) Maintain a repository of cell lines, perform polymerase assays, provide serological support services, and examine material in the electron microscope for the presence of tumor viruses.

Major Findings: A. Isolation and Characterization of Type C RNA Tumor Viruses: 1) In an attempt to isolate the human type C RNA virus, cultures of human tumor wart placenta and chord cells were initiated both on plastic and glass with and without prior treatment with rat-tail collagen. Actively growing cultures were then treated with IDU, dexamethasone, hydrocortisone and insulin then overlaid with cells from human and other species. Sudden spurts of activity always disappeared within 1 or 2 subcultures and no typical type C RNA virus was evident by electron microscopy. 2) Substantial amounts of tritium-labeled particles of density in the 1.16 g/cc were obtained in supernatants of human placental cell cultures, but there was no clear evidence of type C particles by EM or RT. No 60-70s RNA was detectable in placental preparations. 3) Of the several human placentae established in culture, two became senescent at passage 15 and 22. 4) Foci of morphologic alteration in placenta cultures treated with 3MC and IDU have been obtained. 5) Human placentae were cocultivated with cells of a variety of species including chimp placentae, but no significant RT activity was detected. 6) A human nonproducer line was developed from foci transformed by KiMSV. The sarcoma genome can be rescued by leukemia virus of mouse or woolly monkey. The rescued sarcoma virus exhibited an altered host range. 7) Several studies are in process to further characterize the human NP cells. 8) Several studies

are also under way using the human NP cells in attempts to isolate endogenous viruses of several species. 9) Simian and horse cells are also susceptible to sarcoma viruses and Rhim is attempting to isolate other NP cell lines. 10) Work is continuing on characterization of the BuDR activated guinea pig virus. 11) Type C viruses can readily be rescued from NIH nude mice by inoculating the mice with cell suspensions of human and animal tumors and reestablishing the resultant tumors in culture. Preliminary results suggest the nude mouse carries N-tropic, B-tropic and xenotropic viruses. 12) EDU did not activate virus in BALB/c cells or the endogenous virus in Fischer rat embryo cells, nor did it interfere with inducibility of IuDR or BuDR. 13) Neurologic disease induction in mice inoculated with various Casitas virus isolates are continuing. 14) One CMV isolate has been made from immunosuppressed mice and is being studied as a possible model for similarly induced CMV disease in humans. 15) Extensive virus isolation procedures and EM studies were conducted on specimens from infectious hamster lymphosarcomas but the etiologic agent(s) has not been identified. B. Diagnostic Testing and Service Functions: 1) EM: 363 specimens were examined for the presence of virus or virus-like particles in collaboration with investigators on this contract, contracts 3240, 3248 and the Project Officer. 2) Cell Repository: 80 cell cultures in flasks, 3 suspensions and 14 frozen vials were distributed to groups within the VCP. 78 cell lines isolated in this laboratory or received from outside were characterized as to presence of type C RNA viruses and mycoplasma, then frozen for future reference and distribution. 3) RT Assay Service: 80-85 assays have been performed per week for this contract and contract 3254. 4) Sera and Virus Diagnostic Testing: 9000 serodiagnostic tests were performed on 4350 specimens. Sendai virus antibody assays were performed on 3108 sera. 5) AHH Assay Service: About 500 individual tissue culture plates were assayed for AHH activity using coded procedures. No problems are anticipated that would prevent continuation of the studies being conducted under this contract.

Significance to Biomedical Research and the Program of the Institute: Important findings regarding the mechanism of action of oncogenic viruses, their effects on cellular growth control mechanism and their possible involvement in natural oncogenesis have been developed by the contractor. A number of investigators have observed type C RNA viruses in human placental tissues; however, it has not been possible to isolate the virus, grow it in cell cultures and characterize the virus. An endogenous xenotropic type C human virus is likely to be responsible for the generality of cancer under natural conditions and the isolation of this virus would provide an important handle for the eventual prevention and control of human cancer. Also, the contractor provides service functions (electron microscopy, serological, etc.) for other contractors in the Virus Cancer Program and to intramural NCI scientists.

Proposed Course: Continue studies outlined in above Objectives Section.

Date Contract Initiated: November 23, 1974

Current Contract Level: $825,917

ST. LOUIS UNIVERSITY (NO1-CP7-0692)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by RNA and DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This research program is aimed at understanding in detail the mechanism of cell transformation by RNA and DNA tumor viruses, applying new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer, and searching for inhibitors of polymerase that may control the expression of cancer specific genetic information.

Major Findings: About 440 human tissues were collected, stored in vapor nitrogen (1100 total), and computerized by the systematized nomenclature of pathology. Nucleic acids were extracted from about 200 and reverse transcriptase analyzed in 94 tissues. The laboratory developed the methodology to label adenovirus DNA, restriction fragments associated with transformation, and herpesvirus DNA to very high specific radioactivity by nick translation in vitro. Analyses of 61 GI and lung tumors for Ad2 DNA sequences gave 11 positives. Of 28 normal tissues, four were positive, two from a patient with cancer of the same organ, and two from colons of patients with diverticulosis. Studies with restriction fragments and with DNA of other adenovirus serotypes will help clarify the role of these viral sequences in human tumors. Several cultured human cancer cell lines contain adenovirus specific information and are being analyzed for "transforming DNA sequences", tumor antigens, and viral mRNA.

Using a specific template-primer, reverse transcriptase was detected at a density of 1.16 g/cc in 50% of human breast cancers, and in 25 to 50% of a smaller number of cancers of the ovary, Hodgkin's disease and lymphomas. Highly radioactive single stranded and duplex DNA probes were synthesized by purified αβ AMV DNA polymerase acting on 70S RNA from type C virus of the baboon, woolly monkey, and gibbon ape, and by the endogenous reaction of purified virions; these were characterized, and used to search for viral sequences in human tumors. Two RNase H enzymes were isolated from purified Harvey and Moloney MSV(MLV), one cellular and one associated with the viral reverse transcriptase. Purified MSV(MLV) reverse transcriptase possesses 70S RNA reading ability and RNase H levels comparable to the AMV enzyme. The α and β forms of AMV DNA polymerase have different modes of RNase H activity; the β subunit facilitates tighter binding to a variety of nucleic acids. Tryptic and chymotryptic maps show that the peptide sequences of the α subunit are present in the β subunit.

Since a subclass of early adenovirus 2 genes are involved in cell transformation, the laboratory has studied the early events during productive infection. Two classes of early viral RNA transcripts were detected, abundant RNA and scarce RNA, the latter presumably precursor to the former. Four size

classes of L and H strand-specific early viral mRNA were isolated and mapped on the viral genome. Studies on processing of viral RNA by methylation disclosed alkali-resistant short clusters of 2'-O Me base sequences in late viral RNA. Fingerprints of early ^{32}P-mRNA were prepared and distinctive RNase T2 stable tri- and tetra-nucleotides were isolated. A 75,000 molecular weight early protein, found in a nuclear fraction that synthesizes adenovirus DNA in vitro, binds to single-stranded DNA, and possesses T antigen activity. A radioimmune assay is being used to study the role of this protein in transformed cells and in human cancers. The 5' and 3' ends of adenovirus 2 DNA have been partially characterized, the terminally repetition sequences fingerprinted, and the composition of pyrimidine oligonucleotides obtained.

Significance to Biomedical Research and the Program of the Institute: Within the Virus Cancer Program sequential scientific activities, which must be conducted prior to the development of a means for the prevention of virus-induced neoplasia in man, include a) detection of the virus or virus product in human materials, b) identification of the virus as a known or new agent, c) selected biochemical characterization of the agent, d) verification of oncogenicity for man. The basic research on the molecular biology of normal and virus infected cells may provide the basis for understanding the mechanism of animal virus infection and carcinogenesis and for developing a rational chemotherapy for viral diseases and cancer.

Proposed Course: The following investigations are planned: 1) The analysis of human cancers for DNA sequences specific for RNA tumor viruses and for RNA-directed DNA polymerase activity, 2) analysis of human tumors for adenovirus and herpesvirus specific DNA sequences, 3) properties of purified RNA-directed DNA polymerase of RNA tumor viruses and their associated RNase H activity, 4) properties and synthesis of virion and intracellular viral RNA and nascent DNA of oncornaviruses, 5) mechanism of virus replication and cell transformation by RNA tumor viruses, 6) synthesis of RNA tumor virus-specific proteins and their regulation - in vivo and in vitro studies, 7) inhibitors of viral and cell DNA and RNA polymerase molecules - an approach to the chemotherapy of viral diseases and cancer, and 9) human adenoviruses - mechanism of virus replication and cell transformation.

Date Contract Initiated: March 20, 1967

Current Contract Level: $1,200,000

SALK INSTITUTE (NO1-CP4-3243)

Title: Interactions Between RNA Tumor Viruses and Other Viral Agents

Contractor's Project Director: Dr. Walter Eckhart

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: To determine whether infection with a DNA virus induces the appearance of RNA tumor virus RNA in infected cells.

Major Findings: Dr. Wade Gibson and Dr. Inder Verma have studied the subunits of avian virus reverse transcriptases, using techniques developed by Dr. Gibson for labeling polyoma virion proteins with radioactive iodine. The structural relationship of the small (α) and large (β) subunits was examined by tryptic peptide analysis. Tryptic hydrolysates of the isolated subunits were compared by two-dimensional separation on thin layer cellulose plates. The results indicate that the α subunit of avian myeloblastosis virus (AMV) reverse transcriptase is structurally related to the β subunit, and that the subunits of the Rous sarcoma virus (RSV) reverse transcriptase are similarly related. There also appears to be extensive amino acid sequence homology between the reverse transcriptases of AMV and RSV. Both α and β subunits can be identified in purified AMV virions. These experiments are described in a paper to be published in the Proceedings of the National Academy of Sciences.

Significance to Biomedical Research and the Program of the Institute: The expression of genes (viral or cellular) causes an alteration of cell growth. If it is possible to understand the factors that govern expression of RNA virus genes in the mouse system (where the molecules involved can be identified), much of that information should be applicable to human cells. Thus, these studies serve as a model for the human situation.

Proposed Course: 1) To characterize the type C viral RNA in uninfected and polyoma-infected 3T3 cells to see whether size changes occur after polyoma infection.

Date Contract Initiated: June 5, 1967

Current Contract Level: No FY75 funds

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP4-3375)

Title: Immunologic Study of RNA Tumor (Type C) Viruses

Contractor's Project Director: Dr. Frank J. Dixon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This project offers a multidisciplinary study of oncorna viruses, with special emphasis on the situation in the New Zealand (NZ) mouse, and the nature of their interaction with their host which may lead to the formation of tumors or to the development of immunologic diseases, or both. The disciplines involved are virology, molecular biology, biochemistry and immunopathology. The immunologic emphasis in this work relating both to the immunopathologic consequences of oncorna virus infection and to the use of immunologic markers of viral presence are perhaps a unique aspect of this proposal. A better understanding of the immunologic consequences of oncorna virus infection is needed if we are to understand viral oncogenesis and host defense mechanisms, and to achieve effective therapeutic and/or prophylactic immunotherapy, and also to avoid the possible immunopathologic complications of such therapy.

Numerous reports have identified immune responses to many persistent viruses, including the oncorna viruses, with associated immunological diseases of varying severity. The manifestations of these immune responses; i.e., antibodies and sensitized cells, can serve as indicators of the presence of such infections and, in the case of oncorna viruses, provides one of the best and at times the only evidence of their existence. The fact that antibody responses to oncorna viruses may be associated with particular immunologic diseases, such as immune complex nephritis, prompts a search for oncorna viruses in patients with these conditions with particular emphasis on placentas from lupus patients. In addition, there is strong evidence linking the oncorna viruses to human immunologic disease and suggesting that nuclear antigens may be present in a particularly immunogenic form as a result of oncorna virus infection. Thus, the pathologic package offered by oncorna virus infection may include tumors for the immunologically unresponsive host and immunologic disease for the most responsive, with appropriate combinations for those in between.

The approaches taken are designed to determine how the virus acts to initiate either tumors or self destructive immune responses, and what factors in the host determine the pathological outcome of such an infection, with the objective of developing methods of preventing or interrupting the infectious process and its consequences.

Major Findings: The 10 major lines of study which have been pursued under Contract NO1-CP4-3375 during the past year are:

1. Neonatal infection of mice and rats with SLV has been found to produce both lymphomas and immunopathologic disorders. In (BALB/c x NZB)F_1 mice SLV induced lymphomas, antinuclear antibodies and immune complex glomerulonephritis in direct proportion to the dose of virus administered. The effect of SLV on neonatal mice of 15 other strains varied considerably, indicating the importance of the genetic make up of the host in determining the nature of the SLV induced disease.

2. An immunologic analysis of the possible role of oncornaviruses in the immunologic diseases of New Zealand mice strongly suggests that New Zealand mice have more than one viral infection and that it is not possible at this time to determine which virus or viruses is responsible for the high levels of viral antigens in these animals.

3. A study of the distribution and metabolism of MuLV p-30 and gp 69-71 in 8 murine strains indicates a far different pattern of metabolism of these two antigens. p-30 tends to be relatively fixed in tissues and slowly released into the serum while gp 69-71 is found in high levels in the serum in urine indicating its rapid release from tissues.

4. The pattern of SLV vertical transmission in 5 murine strains varies greatly from strain to strain. Both intrauterine and mammary modes of infection of the offspring are found. Only the NZB mouse, presumed to be the natural host of SLV, did not transmit a productive viral infection from infected parents to offspring, suggesting a unique resistance in the NZB neonate.

5. It has been found that the GIX antigen of thymocytes thought to be a genetically determined alloantigen of the mouse is identical to the gp 69-71 of MuLV. This observation lends strong support to the concept that at least part of the MuLV genome is inherited in a mendelian fashion.

6. The pattern of gp 69-71 on the cell surface during differentiation differs considerably in several mouse strains. BALB mice have little or no gp 69-71 at any time. 129 mice have this antigen on their thymic cells but not on bone marrow or spleen cells, AKR and NZ mice have a large amount of gp 69-71 on thymus, bone marrow and spleen cells.

7. In order to determine the precise role of different viruses in the oncogenic and immunologic diseases of the various mouse strains we have begun the isolation of gp 69-71 from several viruses including Rauscher, SLV and xenotrope in order to have specific immunologic reagents.

8. A study of the infection of adult mice with murine oncornaviruses indicate a tremendous increase in permissiveness with relatively small doses of radiation.

9. Placentae from humans with lupus erythematosus have been shown to contain type C virus particles and on cocultivation with primate or rat fibroblast generate reverse transcriptase activity and also can activate virus formation from nonproducer cells.

10. In keeping with the difference in oncogenic potential of thymocyte derived and fibroblast derived oncornaviruses we have found a significant difference in the buoyant density and polymerase activity of oncornaviruses derived from murine thymocytes and fibroblasts in culture.

Significance to Biomedical Research and the Program of the Institute: This program is relevant to the goals of the National Cancer Institute in relation to 1) determination of cause, 2) early detection of risk, and 3) eventual effective prevention of cancer. The etiological association between type C RNA viruses and cancer has been firmly established in chickens, mice, hamsters, rats and cats and is strongly implicated in cows, gibbon apes, woolly monkeys and baboons as well. In addition, the particle has been seen in human placentas to a titer suggesting that it is universally present. Expression of this apparently universal oncogenic potential, however, is dependent on the immunologic responses of the host. Although these immunologic controls are well recognized, the mechanisms are poorly understood. Characterization of the viruses, viral antigens and host cell responses will provide important insights into the etiological role of the viruses under varying immunologic conditions, and the means of detecting and interfering with their oncogenic properties through the use of viral vaccines.

Proposed Course: The project will continue to focus on several interrelated aspects of the oncogenic and immunopathologic consequences of oncorna virus infection with particular emphasis on the situation in NZ mice. 1) The possible role of the oncorna virus(es) obtained from NZB mice in the genesis of spontaneous tumors and immunologic diseases of the NZ mice. 2) Using the

NZB virus(es) as a model, attempt to find if and how in vivo infection of a variety of murine and rat strains with this agent cause tumors and immunologic diseases. 3) Isolate and characterize the glycoprotein surface antigens of oncorna viruses and use this antigen as one of the yardsticks in evaluating the host-virus relationship. 4) Analyze the number and nature of the viruses produced by continuous thymoblast lines derived from NZB and related mice. 5) Correlate a variety of virion coded or induced phenotypic markers of infected lymphoblasts with their oncogenic or immunologic potential in vivo. 6) Study the incorporation of exogenous nucleic acids into oncorna and other RNA viruses and attempt to determine whether this process plays a role in the induction of "autoimmune" responses following infection with these viruses. 7) Search for human type C viruses in the placentas of humans with lupus erythematosus.

Date Contract Initiated: June 29, 1972

Current Contract Level: $656,502

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF and CHILDRENS HOSPITAL OF LOS ANGELES (NOI-CP5-3500)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
Dr. Robert M. McAllister (Childrens Hosp.)
Dr. Brian E. Henderson (USC)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To mount a multifaceted, highly interrelated program designed to determine the roles of viruses, physical and chemical carcinogens, and other factors in the etiology of human and animal cancer in a natural urban ecology. These studies are carried out at USC School of Medicine and at Childrens Hospital of Los Angeles.

Viral Studies: Human, pet and feral animal cancer and fetal materials are under intensive study for RNA tumor virus expression, utilizing all the modern in vitro as well as in vivo test systems. Extensive field studies and procurement efforts provide large numbers of tissues derived from cancer patients, genetically defective individuals, and spontaneous and therapeutic abortions. These materials are utilized for in vitro and in vivo biological studies and are subjected to serological, immunological, biochemical and electron micrographic analyses designed to detect, isolate and characterize the RNA viruses and virus-specific antigens associated with naturally occurring animal and human neoplasms.

Epidemiological Studies: This program is providing, through hospital record surveys and community questionnaire surveys, up-to-date information of the natural occurrence of human cancer as it may be influenced by genetic, viral,

environmental or other factors, including exposure to variable smog components in differing ecological areas of Los Angeles County, industrial and household carcinogens, and pets with and without cancer. Other factors such as occupation, aging, genetic defects, smoking, hormone therapy, and immunosuppressants are being studied using classical epidemiological methods combined with virological and serological surveillance.

Environmental Studies: This program is concerned with monitoring focal environmental areas for levels of carcinogens and other air pollutants. Materials collected are characterized and supplied to laboratories at USC as well as to NCI and other VCP contract programs; e.g., Contract NO1-CP4-3240, for studies to determine the carcinogenic activities of such pollutants in tissue culture and in animals.

Major Findings: Human tumor cell lines have been established and characterized from 20 lymphomas, 2 sarcomas and 2 bladder carcinomas. The lymphoma lines are of B cell origin and contain EBV nuclear antigen. Extensive efforts to activate or rescue a human type C virus from these cell lines and from human placenta cultures remain unsuccessful. One human lymphoma cell line has apparently been non-productively infected with GaLV and a type C virus induced from these NP cells by BuDR. The in vitro host range of the baboon virus (BKD) was similar to that of RD-114 and the p30 antigen induced in indicator cells could be assayed equally well in CF tests by antisera to p30's of RD-114 or BKD. Sera from 20 children with cancer did not contain neutralizing antibody to MSV (BKD) or MSV (GAL). Transformation of mammalian cells has been accomplished with DNA extracted from cells productively infected with RD-114, RD-FeLV and RD-GAL. Efforts to detect transfection of provirus in human sarcoma and leukemia cell DNA are thus far negative.

In wild mice an excellent correlation was found between prevalence and titer of spleen CF gs antigen detection at weanling age and susceptibility upon aging to naturally occurring lymphoma, carcinoma and paralysis. Transmission and in vivo neutralization studies confirmed the prime etiologic role of the indigenous type C virus in the lymphomatous and paralytic diseases. Prevention of the lymphomatous and paralytic diseases and control of the increased level of type C virus expression in the susceptible colony of wild mice (LC) was accomplished at the genetic level by cross-breeding with the C57/Bl/Sn inbred mouse. Efforts at natural disease prevention and prevention of chemical carcinogenesis in wild mice using specific viral immunotherapy are also in progress. The in vitro host range of wild mouse type C virus isolates is generally pantropic. End-point dilution studies suggest that the respective isolates are composed of a homologous population of particles with dual tropism (SC-1 and Sirc) and not of separate mixtures of ecotropic and xenotropic viruses. Specific phosphoproteins have been demonstrated as virion structural components of mouse and cat type C virus.

Ecotropic type C viruses have been isolated and characterized from early subcultures of Sprague Dawley and Fischer rat embryo cells.

The Cancer Surveillance Program now has on computer file approximately 75,000 records of malignancy in Los Angeles County from 1972-1974. Lung and other

cancer incidence rates in Los Angeles County are being correlated with particulate air pollution levels at 39 sampling stations. There were no striking differences except in the south central area, where an increased death rate from lung cancer was detected in Caucasian males. Twenty-one cases of benign liver adenoma in women of child-bearing age and almost entirely associated with the "pill" have been identified in Los Angeles County. Preliminary studies of familial risk to breast cancer revealed an apparent relationship to a genetic abnormality in hormone excretion.

Careful design and statistics in an epidemiological study virtually ruled out a human virus as an important cause of breast cancer; at the same time, the study pointed towards ruling in or out new approaches, one of which is a precise design which involves certain specific hormones. New tumor cell lines were apparently isolated that were of special interest. The fluorescence focus assay for wild type viruses has now been developed and should be much superior to the XC test and the transformation focus assay using pseudotypes which pose problems for wild mouse viruses. The formalin killed vaccine is poor in the Casitas (high leukemia) wild mouse population, but killed plus live virus offers possibilities. Efficient techniques have been developed for separating cells in lymphomas which leave them viable. New baboon virus isolates have been made that are indistinguishable; some were tested (negative results) along with RD-114 in an effort to detect neutralizing antibody in 25 children with a variety of tumors. Transfection experimental protocols have apparently now been perfected with RD-114 so that it is known that 160,000 viral gene copies are required per transformation event, which is 10 - 100X better than that achieved for adenoviruses and about equivalent to that achieved for SV_{40} DNA. It appears SS DNA can transfect as well as DS DNA.

Significance to Biomedical Research and the Program of the Institute: This program searches for causes of human, pet and other animal cancers in a natural ecology, utilizing 1) experimental animal systems, 2) basic viral and chemical carcinogenesis studies, and 3) epidemiological profiles of cancer incidence in Los Angeles Area humans and animals in relation to exposure to environmental carcinogens.

Proposed Course: Continuation of 1) efforts to rescue a human candidate type C virus and eventual development of vaccines for trials in cancer patients, 2) studies of the type C virus isolated from a feral house mouse which is responsible for naturally-occurring early lymphoma incidence and amyotrophic lateral sclerosis-like paralysis in the colony under study and in other mice as well under experimental conditions, 3) epidemiological studies of factors influencing cancer incidence and type, 4) characterization of air pollutants, and 5) procurement, growth and distribution of human and animal materials to collaborating groups within the Viral Oncology Program.

Date Contract Initiated: June 26, 1968

Current Contract Level: $2,561,932

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NO1-CP5-3518)

Title: Conditional Lethal Mutants of RNA Tumor Viruses

Contractor's Project Director: Dr. Peter K. Vogt

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: 1) To continue the isolation of temperature-sensitive mutants of avian sarcoma and leukosis viruses; the goal of this effort is to build a collection of about 300 well-characterized mutants of different avian tumor virus subgroups. Special emphasis is being placed on mutants which are affected in their transforming ability. 2) To characterize each newly isolated mutant with physiological and genetic techniques. 3) To determine the number of viral genes involved in transformation and identify the products of these viral genes.

Major Findings: The isolation of temperature sensitive mutants has been continued. Twenty-one new isolates have been obtained in the past six months.

Several avian sarcoma virus temperature sensitive mutants have been used to transform NRK and Microtus agrestis cells. These temperature sensitive mammalian cells will now be fused to test for complementation of transforming functions in the absence of virus synthesis. In collaboration with Dr. Scolnick of the National Cancer Institute we will also include NRK cells transformed by temperature sensitive murine sarcoma viruses in these complementation tests.

Several multiple mutants have been segregated into single mutants by recombination. Of particular interest among the single mutants is one affecting an early transforming gene and two replication mutants, one in RNA synthesis the other interfering with virus assembly and budding.

As a prerequisite for studies of intracellular viral proteins in mutant infected cells we have examined the processing of the major polyprotein precursor of intraviral structural proteins in the cell. This precursor is normally cleaved to p27, p19, p15, and p12, but cleavage is inhibited in the presence of glucosamine.

Significance to Biomedical Research and the Program of the Institute: The work on avian sarcoma virus ts mutant has provided and will provide detailed information on the function of individual viral genes, on their timetable of action and their location on the viral genome. Of particular value will be the characterization of viral transforming genes, their number, position on the genome and sequence of activity. Eventually, this work should lead to the identification and isolation of the primary gene products of transforming genes, the hypothetical transforming proteins. The characterization of such proteins will be a major step towards understanding viral carcinogenesis.

Proposed Course: Assuming 50 complementation groups in RNA tumor viruses, one would need about 200-300 mutants randomly distributed over the genetic map to achieve the degree of map saturation required for a rough genetic definition of individual viral genes. The work, therefore, will proceed with the isolation

of 200-300 mutants which will require 2 to 3 years. Physiological and genetic studies will be conducted in parallel with mutant isolation.

Date Contract Initiated: October 15, 1971

Current Contract Level: $250,000

STANFORD UNIVERSITY (NO1-CP4-3228)

Title: Virologic, Biologic and Immunologic Characterization of Hodgkin's Disease and other Human Malignant Lymphomas

Contractor's Project Director: Dr. Henry Kaplan

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: 1) Collection of Hodgkin's tissue and serological samples for study. 2) Cultivation of human lymphoma tissue in vitro. 3) Biological characterization of the established cell lines and clones derived from them. 4) "Rescue" and characterization of viral isolates from the established lymphocytic lines. 5) Immunological characterization of the cells in relation to the host in terms of autoimmune responses.

Major Findings: Using the techniques previously developed for the establishment in vitro of tumor cells from three patients with diffuse histiocytic lymphomas cell lines have now been established in suspension culture from several additional patients with histiocytic lymphomas, an American Burkitt lymphoma, a B-lymphocyte lymphoma, and a Sezary syndrome variant of mycosis fungoides. Some of these cell lines have also been adapted to clonal culture in agar.

The initial requirement of human lymphomas in vitro for a fibroblastic feeder layer has been firmly documented. Preliminary studies with clonal cultures of the lymphoma cells on agar suggest that a diffusible growth factor is elaborated by the feeder layer.

Several of these lymphomas, as well as their established cell lines, and other lymphomas which have not yet been grown in vitro, have been successfully heterotransplanted by intracerebral inoculation into nude mice. In their morphology and degree of differentiation, the heterotransplants closely resemble the corresponding original human lymphomas in situ. Some of the heterotransplants have secondarily yielded cell lines in vitro.

The histiocytic lymphoma cell lines and one American Burkitt lymphoma cell line are negative by the EBNA test and thus appear to lack the Epstein-Barr virus (EBV) genome, despite the fact that some of the patients from which they were derived had EBV-positive serologies and that, in preliminary tests, the cell lines have been susceptible to laboratory infection with EBV.

Electron microscopy revealed virus particles with type C morphology budding from the plasma membranes of some of the secondary cell lines established from

nude mice heterotransplants. Pelleted supernatants of these cultures had reverse transcriptase activity and were infectious for other, previously virus-negative cell lines, as indicated by the induction of reverse transcriptase activity. Preliminary data suggest that these type C virus isolates are endogenous mouse xenotropic viruses.

Antibody raised in rabbits against one of the primary histiocytic lymphoma cell lines, SU-DHL-1, and sequentially absorbed with pooled normal human spleen tissue, pooled buffy coats from normal human peripheral blood, autologous fibroblasts, and autologous, tumor-free spleen tissue, yielded positive reactions in indirect immunofluorescence tests with SU-DHL-1 and other histiocytic lymphoma cell lines, suggesting that these cells bear a common (tumor-specific or carcinoembryonic) antigen.

Several of the cell lines have now been characterized by transmission and scanning electron microscopy, light microscopy, specific histochemical staining reactions, T- vs. B-lymphocyte surface markers, and karyotype.

Quantitative studies of peripheral blood lymphocytes (PBL) from patients with untreated Hodgkin's disease have revealed an absolute lymphocytopenia in about 25% of cases. Percentages of B-cells (measured by surface Ig) and T-cells (measured by cytotoxic assay) were normal, even in lymphocytopenic cases. However, functional deficiency of a subpopulation of T-cells was revealed by the fact that the percentage of E-rosette-forming cells was significantly lower than the total T-cell population detected by the cytotoxic assay. Incubation of PBL in fetal calf serum (but not human serum) increases the proportion of rosette-formers to the cytotoxic level, suggesting that the E-rosette receptor site(s) on some T-cells are reversibly masked by a serum factor. Preliminary data indicate that E-rosette formation by PBL from normal donors may be inhibited by incubation with a partially purified ferritin fraction from the tumor-involved spleens of patients with Hodgkin's disease; the inhibition is reversible after reincubation in fetal calf serum. Treated Hodgkin's disease patients in long-term remission reveal an absolute T-lymphocytopenia, a B-lymphocytosis, and total lymphoctye counts in the normal range. Correlations with in vivo and in vitro tests of cell-mediated immune response have been concurrently studied.

Partial success has been attained in adapting the dinitrochlorobenzene (DNCB) skin test to in vitro assay, using DNCB coupled to dimethylsulfoxide (DMSO)-treated erythrocytes or buffy coat leukocytes.

Significance to Biomedical Research and the Program of the Institute: This project will provide a source of Hodgkin's and other human tumor materials for cultivation and for virus isolations. If antigens specific for the Hodgkin's cells (Reed-Sternberg) can be detected, this would have major implication not only for etiology but also for diagnosis, for the detection of persistent or recurrent foci of disease, and perhaps even for the immunotherapy of Hodgkin's disease.

Proposed Course: 1) Initiate co-cultivation studies with the cell lines already established, seeking to detect the presence of occult viruses. 2)

Establish other lymphoma cell lines in vitro, in particular from additional patients with Hodgkin's disease. 3) Additional studies of the nature of the immune deficit of patients with Hodgkin's disease and other malignant lymphomas will be carried out in vitro with particular emphasis on correlation of the rosette test with the response of peripheral blood lymphocytes to PHS and in the mixed lymphocyte reaction.

Date Contract Initiated: April 1, 1974

Current Contract Level: $245,000

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP4-3241)

Title: Study of Virus-Induced Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To investigate the differences between the structure of the surface of normal cells and of cells transformed by viral and non-viral carcinogens by studying the differential binding of plant lectins.

Major Findings: Glycopeptides from the surface of clones of hamster embryo cells were examined at various intervals after infection with polyoma virus. Two types of transformed cells were examined: (i) clones that showed delayed transformation or an initially low tumorigenicity, and (ii) clones that were rapidly transformed showing an initially high tumorigenicity. The glycopeptides were removed from the cell surface by trypsin and, after Pronase digestion, were examined by filtration through Sephades G-50. With delayed transformation, a specific group of glycopeptides was increasingly evident over an 85-day period as the cells showed phenotypic properties of transformation and the ability to form tumors. In the other series, all but one clone of hamster embryo cells showed rapid transformation after infection with polyoma virus. This clone was less tumorigenic and showed little of the specific glycopeptides. In all cases of delayed or rapid transformation examined, the specific group of glycopeptides increased proportionately to the ability of the cells to form tumors. All of the cells derived from progressively growing tumors formed by injection of these transformed hamster cells into adult animals showed an abundance of this group of glycopeptides. These results suggest that specific surface membrane glycopeptides accompany viral transformation and tumorigenesis.

Significance to Biomedical Research and the Program of the Institute: Compounds that interact differentially with the surface membrane of normal and tumor cells are of value in elucidating the chemical nature of the differences in the surface that are associated with cell malignancy. They are also of potential value for tumor chemotherapy.

Proposed Course: The contract was terminated on October 21, 1974.

Date Contract Initiated: April 22, 1969

Current Contract Level: No FY75 Funds

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NO1-CP3-3250)

Title: Extraction and Characterization of Virus-Induced Transplantation Antigen and Rescue of Virus from Sarcomas and Leukemias

Contractor's Project Director: Dr. Anthony J. Girardi

Project Officer (NCI): Dr. James T. Duff

Objectives: 1) Studies on attempts to isolate viruses from human sarcoma and leukemia patients. 2) Localization of the integrated SV40 genome on chromosomes of transformed human cells.

Major Findings: Human leukemia cells were fused with human sarcoma cells, chemically induced, and evaluated for release of infectious focus-forming virus on five target cell types. No viruses were detected when peripheral WBC of 16 cases of leukemia were fused with a variety of human sarcoma cells resulting in 25 separate combinations which were tested on four or five target cells types for each combination. Furthermore, no gs antigen cross-reactive with murine gs antigens were seen in the fused cultures or in the target cells examined after inoculation.

Hybrid cell clones between mouse cells and two different SV40-transformed human cells showed concordant segregation of SV40 T antigen activity, rescue of defective SV40, expression of SV40 TSTA with human chromosome C-7. These findings indicate a linkage relationship between SV40 genome and this particular chromosome. No other human chromosome showed concordant segregation with these functions. Subclones of positive clones which lost the C-7 chromosome, lost expression of these virus-induced activities.

Significance to Biomedical Research and the Program of the Institute: In order to establish the viral etiology of human cancer, it is essential to pursue new techniques and methodology for the isolation of a potential candidate human tumor virus. These studies are attempts to isolate this elusive agent. The identification of the integration sites of SV40 hybrid genomes in transformed cells and the determination of their linkage relationship with other chromosome markers may explain the mechanisms of virus-induced transformation.

Proposed Course: The contract was terminated on September 30, 1974.

Date Contract Initiated: February 1, 1971

Current Contract Level: No FY75 funds.

UNIVERSITY OF WASHINGTON (NO1-CP3-3372)

Title: Studies on Tumor-Specific Transplantation Antigens

Contractor's Project Directors: Dr. Karl Erik Hellström
Dr. Ingegerd Hellström

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To detect and characterize tumor-specific antigens, plus serum-mediated and cell-mediated immune responses to these tumor-specific antigens, in human tumors.

Major Findings: Cytotoxic effect of lymphocytes from pregnant mice on cultivated tumor cells. Specificity, nature of effector cells, blocking by serum. Lymph node and spleen cells from pregnant mice were found to be cytotoxic to cultivated syngeneic tumor cells. Both the cytotoxic lymphocyte effect and the blocking serum activity were seen in the first pregnancy and lasted for at least one month subsequent to delivery. Thus, serum from pregnant mice could "arm" control lymph node cells and produce a lymphocyte-dependent cytotoxic effect on neoplastic, but not on control, target cells.

In studying the cytotoxic effect of lymphocytes from multiparous mice, the Hellstroms demonstrated that "embryonic" tumor antigens elicited high titered blocking serum factors, and that mice with transplantation resistance to chemically induced sarcomas often had blocking factors to embryonic antigens. They found, further, that the blocking effect of lymph node cells from multiparous mice could be removed from serum by absorption with tumor cells or with cells from regenerating liver.

Generally, the large volume of data developed in several systems support the following conclusions: (1) the poor ability of embryonic antigens to evoke a tumor specific transplantation resistance is due to their great capacity to form and to act as blocking factors, abrogating lymphocyte mediated, cytotoxic reactions; (2) transplantation tolerance may be maintained by an active immunological process and does not occur solely as the result of a deletion or irreversible suppression of potentially reactive lymphoid cells during neonatal life; (3) allograft tolerance may represent a defect of immune amplification.

In summary, work during the past contract year supports the Hellstrom's original observations defining serum blocking factors which play an important role in facilitating tumor growth in vivo; the ability of tumors to induce the formation of such factors may be of fundamental importance for their escape from immunological control. This evidence is further strengthened by the observation that "unblocking" antibodies are often found in animals whose tumors have regressed.

Significance to Biomedical Research and the Program of the Institute: This work represents definition and analysis of the immune response to tumor-associated antigens. The data developed will be directly applicable in approaches to prevent, treat and better diagnose human tumors by immunological means.

Proposed Course: Studies will be continued on the interaction between lymphocyte and serum factors in animals and patients with the ultimate objective of applying findings to cancer prevention and treatment by immunological means.

Date Contract Initiated: April 14, 1969

Current Contract Level: $115,956

CONTRACT SUMMARY REPORT

5. TUMOR VIRUS DETECTION SEGMENT

Human Studies

Studies on terminal deoxynucleotidyl transferase (TdT) in humans have shown that this enzyme is an extremely good marker for cells from acute lymphoblastic leukemia. Normal people have the enzyme only in their thymocytes and, at low levels, in the bone marrow. Three forms of the enzyme have been identified: two forms which are both in thymocytes as well as in acute lymphoblastic leukemia cells and one form which has thus far been found only in the bone marrow. (Contract #33348)

Experiments were carried out on Raji cells, (which contain EBV DNA but cannot be stimulated to produce Epstein Barr virus by all known techniques). The results showed that the EBV DNA sequences banded in the position of cell DNA (density 1.70 g/ml). EBV DNA was also detected at the density of 1.707 g/ml when a sheared DNA preparation was used. Similarly, alkali denaturation resulted in the appearance of EBV DNA sequences in the density position between the cellular and viral DNA. Based on these findings it is suggested that EBV DNA may be present as fragmented molecules integrated into Raji cellular DNA. (Contract #33310)

Distamycin A inhibits the translation of some EBV genetic information in Burkitt lymphoblasts. The greatest inhibitory effect occurs in the synthesis of peptide number II (the capsid protein) and peptides III, IV and V (the glycoproteins). In P_3HR-1 cells, distamycin A prevents the appearance of viral capsid antigen (VCA) while early antigen (EA) is not affected. These results indicate that distamycin A, which binds to A-T sequences in the DNA and inhibits transcription by DNA and RNA polymerases, can selectively inhibit the transcription of the mRNA which contains the information for VCA (=peptide II). In this respect EBV DNA differs from HSV DNA since distamycin A does not prevent the synthesis of capsid peptide II in HSV infected cells. (Contract #33310)

In addition to being able to transform hamster cells, the human papovavirus, BK, can also transform a marmoset, diploid, fibroblast cell line. The efficiency of BK virus transformation at any multiplicity is higher for hamster cells than for the marmoset cells. The BK transformed marmoset cells grow in soft agar and reach a saturation density 4-6 times greater than untransformed marmoset cells. Further, although normal cells reach a stationary phase in culture and remain stable, the transformed clonal lines overgrow due to loss of contact inhibition with progressive cell detachment. (Contract #43318)

Co-electrophoresing the polypeptides of BK virus with those of SV40 and polyoma virus showed that the major virion protein VP1 of BK virus was smaller than that of SV40 and even smaller than that of polyoma virus. VP3 of BK virus and of SV40 were identical in molecular weight. There was little similarity between the tryptic digests of VP1 of SV40 and BK virus and none at all between those of VP3 of the two viruses. However, there was substantial

similarity between VP4, VP5 from the two sources consistent with the claim that these may be histones, coded for by the cell genome. (Contract #43318)

Urine samples were studied from: 1) 23 children with leukemia or solid tumors, most of them in remission, undergoing chemotherapy as outpatients; 2) 9 cancerous adults who were hospitalized and heavily immunosuppressed; 3) age-matched normal children and adults. The frequency of BK virus-specific antibodies was significantly higher in the urines of cancer patients both by the HAI and FA-IgG tests. Aggregates of papovavirus particles were found in 6 of 41 urines from cancerous patients and in none of 21 normal urines. The 6 positive urines came from three patients - 2 adults and one child. Two out of 3 urines of patient #1, all 3 of patient #2, and 1 out of 2 of patient #3 had particles. The duration of excretion (a minimum estimate) was two weeks in patient #1 and three and a half weeks for patient #2. Patient #2 excreted very large numbers of particles. All urine samples of patient #2 and at least one of the other two contained BK virus-specific antibodies. (Contract #43266)

Biological properties of a strain of cells derived from a brain biopsy of a human patient with progressive multifocal leukoencephalopathy were investigated. The patient had a high antibody titer to SV40, and SV40 was isolated from the brain cells. The brain cells were epithelioid in shape. They grew into contact inhibited monolayers and could be subcultured approximately 30 times. Thirty percent of the cells at the 11th passage were diploid and the remainder aneuploid. Although SV40 could be readily rescued from the cells, expression of SV40 T antigen was restricted to the growth phase of the cells and was not detectable in confluent monolayers. Cells did not have V antigen by immunofluorescence, but small amounts of free virus were recovered from sonicated cell preparations. High and low molecular weight DNA extracted from the cells were infectious but most of the infectivity was associated with the high molecular weight fraction. (Contract #43266)

In liquid hybridization experiments with an excess of human cellular DNA the 30S Gross Leukemia Virus RNA subunit (obtained by heat denaturing 60-70S RNA) hybridized to the same extent (2-3%) with DNA from leukemias, solid tumors, normal adult spleens, or four of seven apparently normal placentas. However, with DNA from two placentas, 10% of the large GLV-RNA subunit hybridized and with DNA from one placenta 46% of the 30S GLV-RNA hybridized. A pool of the four GLV-RNA subunits of intermediate size (9S-25S) hybridized significantly (7%) only to DNA from the same three human placentas which hybridized the most with the 30S subunit. The smallest GLV-RNA subunit (4-5S) hybridized equally (11%) to DNA from all neoplastic or normal human tissues. (Contract #33283)

DNA from normal and neoplastic human tissues contain sequences complementary to the RNA of a Woolly monkey oncornavirus (SSAV-1) produced in human rhabdomyosarcoma cells. An average of 43% of the viral 50-60S RNA hybridized with DNA from four different types of neoplastic tissues (bladder carcinoma, hystiocyticreticulosis, adenocarcinoma and acute myelogenous leukemia). DNA from the spleen of a patient who succumbed to generalized atherosclerosis, but in whom at autopsy a cortical renal adenoma was noticed, hybridized 58%

of the viral RNA. An average of 23% of the SSAV-1 RNA hybridized to DNA from two normal human placentas while 50% of the SSAV-1 RNA hybridized to DNA from SSAV-1 producing human tumor cells. (Contract #33283)

Continuous lines of lymphoid cells taken from patients with leukemia were, unlike lines from healthy donors, unable to grow in the presence of cystathionine, and had markedly reduced activity of the enzyme, cystathionase, which cleaves cystathionine to α-ketobutyrate and cysteine. For several murine and rat lymphoma lines tested, this seemed also to be true. An improved assay for cystathionase was developed, and the use of this as an indicator of transformation of a hematopoietic cell is being investigated. (Contract #43269)

Evaluations performed on a group of 12 adult patients with leukemia and lymphoma, at the time of diagnosis, following induction chemotherapy, after the completion of intensified therapy and then monthly for a period of 6-12 months showed the following results: The absolute numbers per mm^3 of T cells are usually either increased or decreased at the onset of the disease. When remission is obtained by induction chemotherapy, percentages of T lymphocytes return to normal values and they tend to stay at the normal level during several months of intensified and maintenance treatment. On the other hand, B lymphocytes are most often normal in absolute numbers at the time of diagnosis, but their percentages drop significantly following radio- and chemotherapy. This decrease is sustained during maintenance therapy. (Contract #33357)

Primate Studies

Two owl monkeys were placed in adjoining cages with two Herpesvirus saimiri infected squirrel monkeys. The 2 squirrel monkeys were excreting virus by the oropharyngeal route beginning 15 days after virus inoculation and virus was also recovered from peripheral blood. The inoculated squirrel monkeys did not develop any disease during the period of observation which lasted over 1 year. One of the owl monkeys after 3 months of contact was positive for H. saimiri. Virus was consistently recovered from the peripheral blood. The presence of virus was detected for 9 months. This animal died after 11 months of uremia. There was no evidence of malignant lymphoma. H. saimiri was recovered from kidney cell cultures of this animal. (Contract #33390)

H. saimiri was able to infect human cell cultures (whole human embryo and human embryonic cell cultures). Although the virus multiplied in these cell cultures, it failed to induce a complete cytolytic infection as observed in owl monkey kidney cells. Virus could be recovered from these infected human cells up to 187 days. After this period no virus could be detected, but by the cell fusion technique virus was recovered at the end of 347 days. (Contract #33390)

H. saimiri was shown to induce malignant lymphoma in New Zealand white rabbits inoculated by the intravenous route. A single inoculation induced lymphomas in a majority of the rabbits. A unique feature observed in the rabbit

lymphomas was the invasion of the skin of the eyelid and nose with lymphocytes or lymphoblasts. This feature has not been reported in studies involving primates injected with H. saimiri. In rabbits that died of lymphoma, there was severe conjunctivitis, nasal discharge and dyspnea. This clinical picture was also not observed in monkeys developing malignant lymphoma. (Contract #33390)

H. saimiri was titrated simultaneously in the cell lines VERO (mycoplasma free) and VERO-N (heavily mycoplasma contaminated) in each case with standard medium (ERM) and with arginine-deficient medium (ERM-Arg). In ERM, the VERO-N gave higher plaque counts than did VERO cells. However, if arginine was omitted from the medium, equally high counts were obtained on both cell types and both were higher than with ERM. Changing the cells to ERM-Arg one or two days after infection resulted in much higher yields of virus than changing the cells immediately to deficient medium or than keeping the controls on complete ERM. (Contract #22068)

Murine Studies

Soluble extracts of mouse cells with Fv-1^n or Fv-1^b gene alleles specifically and reciprocally inhibit infection of B- or N-tropic mouse leukemia viruses in permissive cell cultures. NB-tropic virus infection was not inhibited by either cell extract. Extracts from Fv-1^- cells did not inhibit infection by the three virus host-range types, but N- or B-tropic virus infection of Fv-1^- cells was inhibited by extracts of the nonpermissive cells, and Fv-1^{nb} cell extracts inhibited both viruses. The inhibitor(s) is relatively stable; activity is lost after 2 hrs at 37° or 30 min at 56°C. The inhibitor(s) was most effective if added two hrs before or within two hrs after infection, did not react with the virus directly, inhibit virus attachment, or inhibit the normal cell functions tested. These results indicate that nonpermissive mouse cells contain a product, possibly determined by the Fv-1 gene which inhibits some early postpenetration event(s) in leukemia virus infection. Incubation of the extracts with DNase, RNase and trypsin have shown that the inhibitory effect is sensitive only to RNase. Further biochemical characterization of the inhibitor(s) indicates it is a macromolecule excluded by G25 Sephadex gel; its stability increases as it is purified by DEAE-cellulose and phosphocellulose chromatography; and it is present mainly in the cytoplasm of the cells (microsome fraction and post-microsomal supernatant). (Contract #20208)

Data on the fate of radioactive label after infection of cells with N- or B-tropic Friend Leukemia Virus showed that virus adsorbs to and uncoats equally well when infecting Fv-1 permissive or restrictive cell types; and that the appearance of viral specific RNA in the infected cell nucleus, and its apparent integration with host DNA also is not affected by the Fv-1 restriction gene. Shortly after infection, nuclear associated labelled virion nucleic acid exists primarily as pure RNA when analyzed on $CsSO_4$ density gradients showing a predominant peak of ρ 1.67 (pure RNA), and a smaller peak at ρ 1.55 (putative RNA-DNA covalently linked). The latter peak is not found in pure virus, is barely detectable in cytoplasmic extracts, and represents virion RNA covalently linked to DNA. At later time points,

^{32}P labelled material appears at ρ 1.44 characteristic of essentially pure DNA. Since all these studies are performed on formaldehyde treated nucleic acid, the peaks at ρ 1.55 and 1.44 most likely represent RNA linked covalently to DNA. (Contract #43380)

The RNAs of four different murine sarcoma-leukemia virus complexes (Kirsten, Friend, Harvey and Moloney) could be resolved into two species: One species had the same electrophoretic mobility as the RNA of helper leukemia virus, the other was smaller on the basis of its higher electrophoretic mobility than the leukemia RNA standard. Preliminary experiments using comparative electrophoresis and fingerprinting of electrophoretically purified RNA components indicate that the large RNA components of sarcoma-leukemia complexes are identical to the RNAs of pure helper leukemia virus. Preliminary experiments indicate that the electrophoretic mobilities of the small RNA components, present in the defective sarcoma-leukemia virus complexes, are different. Kirsten sarcoma virus contains the RNA with the lowest electrophoretic mobility and Friend-, Harvey- and Moloney sarcoma virus, in this order, contain RNAs of higher mobilities. Comparative fingerprint studies of the electrophoretically purified small RNA components, of two of the four transforming viruses (Kirsten and Moloney) indicate that they are little related to each other. However, preliminary studies suggest that the fingerprint patterns of the small, presumably transformation-specific, and the large, probably helper leukemia-specific, RNA components of the Harvey sarcoma-leukemia virus complex are related. (Contract #43212)

Most cellular DNA polymerases from several sources (e.g., nonactivated AKR cells, normal mouse spleen) have no affinity for poly(Um)-Sepharose columns at low salt concentrations. Reverse transcriptase (from RLV, AKR virus, activated AKR cells, or RLV-infected spleens) binds to the column and can be eluted with an NaCl gradient, leading to separation of the viral enzyme from most cellular DNA polymerases. (Contract #20208)

Pretreatment with poly(Am) significantly inhibits the induction of sarcomas by Moloney sarcoma virus in newborn mice. Treatment of the mice four hours before MSV inoculation was much more effective than one hour pretreatment. Inoculation of the drug at the time of virus infection, even when subsequent doses were given after infection, was not effective. High virus concentrations could overcome the inhibitory effect of poly(Am). (Contract #20208)

Normal $B6C3F_1$ mouse serum contains antibodies specific for endogenous murine leukemia virus directed mainly against the viral envelope proteins gp70, gp45 and p15. The antigens recognized by autogenous immune sera do not differ with respect to age of the animals, nor are they significantly different in sera from various strains of mice (BALB/c, C57BL/6, C3H/Anf). The 19S antibody fraction of $B6C3F_1$ serum reacted with gp70, gp45 and p15. In contrast, the 7S component reacted only with p15. Against BALB:virus-2, a xenotropic endogenous mouse type C virus, 19S and whole serum, but not the 7S fraction, possessed neutralizing activity. (Contract #20208)

Mice from a wide variety of inbred strains formed natural antibody against endogenous MuLV. Mice of some strains (I, SJL/J, NZB, and NZW) produced

high titers of antibody while mice of other strains showed either intermediate titers (DBA/2, C3H/An, HSFS, 129, GR, and AKR) or relatively low titers (C58, C57L/J and PL/J) of viral antibody. In general, older mice contained higher titers of antibody; however, there were many exceptions to this rule. These antibodies occurred in the γM, γG, and γA immunoglobulin classes, although the highest titer antibodies were of the γG_1 and γG_2 types. Mouse antibodies were directed against four of the six major viral proteins, gp70, gp45, p15 and p10. A demonstrable immune response to proteins p30 and p12 was not observed. The γG_1 and γG_2 immunoglobulins of an NZB serum precipitated gp45 and p10, whereas the γM immunoglobulins from the same serum precipitated gp45, p15, and p10. This was in contrast to the serum of an NZW mouse, where antibodies (in all immunoglobulin classes) were directed against proteins gp70, gp45, and p15. (Contract #53515)

Glucocorticoids increase both murine mammary tumor virus (MTV) virion, and viral RNA production in cultured GR cells. GR cell glucocorticoid receptors are indistinguishable from those of S49 cells based on their sedimentation and DNA-binding properties. S49 cells contain MTV RNA in quite significant quantities. Nevertheless, S49 cells cannot be induced by the glucocorticoids to increase the MTV RNA content although these cells have other functions clearly under hormonal control. (Contract #33332)

A mutant of polyoma virus has been found, which is cold-sensitive for transformation of BHK cells. The permissive temperature is 38°C and the nonpermissive, 32°C, with a differential between the two temperatures of approximately 5 to 7 fold. This differential remains consistent over a dose dependent range of transformation from 5 to 60 pfu/cell. The mutant appears to be temperature sensitive not only for the establishment of transformation, but also for maintenance. For rat fibroblast cells, there is no cold sensitive transformation by this mutant. (Contract #43318)

The polyoma host range mutant, NG-18, screened originally for its ability to grow in polyoma-transformed cells, has now been shown to grow in a variety of other cell types. Among cells which are substantially permissive for the growth of NG-18 are: phenotypic revertants of polyoma-transformed cells, type C RNA virus transformed and/or producing cells, cells which become transformed upon infection with leukemia virus, primary baby mouse kidney epithelial cells, and primary mouse embryo fibroblasts. Among cells which are poor hosts for the growth of NG-18 are: all 3T3 cell lines, SV-40 transformed 3T3 cells, spontaneously transformed 3T3 cells, radiation and chemical carcinogen transformed 3T3 cells, and late passage mouse embryo fibroblasts. Similar results have been obtained using three other independently isolated host range mutants of the same type as NG-18. The permissive state thus does not require a cell-associated polyoma genome as the basis for complementation of growth for this class of host range mutant. (Contract #43299)

Multiple forms of the murine enzyme, terminal deoxynucleotidyl transferase (TdT) have been analyzed and the distribution of the enzyme among the various types of murine thymocytes has been characterized. Most of the enzyme is sensitive to cortisone treatment and those thymocytes resisting cortisone

treatment have largely that form of TdT which is ordinarily less that 10% of the total. The distribution of the two terminal transferase activities is a valuable parameter in distinguishing thymocyte subpopulations, and perhaps in establishing a developmental sequence within the thymus. Thus far all TL (Thymus Leukemia) antigen positive tumor lines carry terminal transferase activity and no TL negative line yet has been shown to have the enzyme. (Contract #33348)

Avian Studies

Reverse transcriptase isolated from avian RNA tumor viruses consists of two poly-peptide chains: an α subunit of 60,000 molecular weight and a β subunit of 100,000 molecular weight. It appears that the β subunit plays a role in the binding of the holoenzyme (α,β) to template primer. (Contract #33348)

When crude extracts of secondary chick embryo fibroblasts infected and transformed by RSV were fractionated, all of the reverse transcriptase activity recoverable from phosphocellulose columns was found in the membranous fraction of the cell, and was further characterized as associated with the plasma membrane suggesting that most of the "intracellular" active enzyme could be in budding virus particles or at least in a state preparatory to budding. The reverse transcriptase activity could not be washed from the membrane fraction by high salt buffer. Furthermore, to assay the reverse transcriptase activity in the membrane fraction required addition of a nonionic detergent (nonidet P40) to break the membrane structure. These results suggest that the activity is found inside of particles. (Contract #33348)

A radioimmunoassay for reverse transcriptase was developed by purifying AMV reverse transcriptase to apparent homogeneity. Both AMV or RSV gave similar curves except that the enzyme in RSV virions was unable to displace all of the ^{125}I AMV reverse transcriptase. It is concluded that the enzymes from AMV or RSV are very similar but they have some type-specific antigenic determinants. Reverse transcriptase from murine leukemia virus (Moloney) has no common antigenic determinants with AMV. 2.5% of the proteins in the AMV virion are reverse transcriptase; this amounts to about 70 molecules of enzyme per virion. (Contract #33348)

The template activity of the 60-70S RNA complex and of the 30-40S subunit RNA species of Rous sarcoma virus (RSV) was tested in a cell-free protein synthesizing system from mouse ascites Krebs II cells. Stimulation of protein synthesis over the endogenous background was about two-fold with 30-40S RSV RNA and about 1.3-fold with 60-70S RSV RNA as template. Analysis by SDS-gel electrophoresis showed that the predominant polypeptide synthesized in vitro in response to 30-40S RSV RNA had a molecular weight of 75-80,000 daltons. This polypeptide could be precipitated by antiserum against the group-specific (gs)-antigens of RSV, although its molecular weight is higher than that of virion-gs-antigen proteins. Analysis of tryptic digests of the in vitro-made protein indicated similarity to tryptic digests from authentic virion-gs-proteins. (Contract #43212)

Analyses of the RNAs of viral recombinants led to the conclusion that recombination among avian RNA tumor viruses involves crossing over. (Contract #43212)

By electron microscopy, it was found that 60-70S RNA of Prague Rous sarcoma virus had an approximate average weight of 6 X 10^6 daltons and contained predominantly two 30-40S subunits held together at many points in a network structure. The size of the 30-40S RNA was determined to be around 3 X 10^6 daltons which is in good agreement with earlier determinations of the molecular weight of 30-40S RNA obtained by other methods. (Contract #43212)

When cellular 4S RNA is mixed with AMV or AKR viral 35S RNA at 55-60°C in a 0.1 M NaCl solution for 16 hrs, specific species of 4S RNAs, demonstrated to be one prominent peak and 3-4 minor peaks by reversed phase chromatography, are found to associate with the viral RNA. After hybridization with cellular 4S RNA, the AMV 35S RNA regains its template activity with reverse transcriptase suggesting that the cellular 4S RNA provides the primer function. $tRNA^{trp}$ constituted 50-60% and $tRNA^{lys}$ constituted 10-20% of the major peak of chicken embryo culture cell 4S RNA which specifically become associated with the AMV 35S RNA in vitro. However, there was no evidence of the presence of $tRNA^{trp}$ or $tRNA^{lys}$ in the 60-70S associated 4S RNA of AKR MuLV or among the specific 4S RNA of AKR cells which hybridize in vitro to AKR-MuLV 35S RNA. (Contract #20208)

In cultured chicken embryo fibroblasts infected with avian sarcoma or leukemia virus, viral DNA (vDNA) synthesis was detected intracellularly within 1 hr after infection, and vDNA integration into cellular DNA within 24 hrs. There was a several-fold increase in the vDNA content of infected cells during the initial phase of virus infection. But, only a part of this newly synthesized vDNA appeared to become covalently linked with high molecular weight cellular DNA. In normal chicken cells, endogenous viral DNA appears to be associated with cell sequences reiterated 1200 times, and each integration unit appears to have a maximal size approximately equivalent to the 35S RNA subunit of the virion. In AMV infected cells, additional viral sequences are found which reassociate as if they integrated adjacent to unique cellular DNA. (Contract #33283)

A kinetic complexity of 3.3 X 10^6 daltons was obtained for the 35S AMV RNA. This value is very close to the estimated molecular weight of a 35S subunit. Therefore, all the 35S subunits are either identical or differ only slightly. The AMV RNA sequences which hybridize rapidly in DNA excess are not restricted to any one region of the AMV 35S RNA. They appear to be randomly distributed over the entire RNA molecule and some are positioned within 200 nucleotides of the poly(A) tract at the 3'-end of the RNA. (Contract #33283)

Infectious DNA able to elicit the production of virus in permissive chick embryo cells (CE cells) can be recovered from transformed CE cells producing either RS-RSV (D) or the ts mutant FU-19 of this virus, and also from nontransformed CE cells producing either RAV 1 or RAV 50. DNA is infectious only when cells are pretreated with DEAE-dextran; the optimal dose is about 100 µg/ml, for 30-60 min before infection with DNA. (Contract #43219)

Cultures of CE cells infected with Bryan strain RSV in the presence of the medium of CE cell cultures transformed by SR-RSV, or of RS2 cell cultures, can increase 2- to 6-fold the number of foci of Rous cells, relative to untreated controls, or cultures incubated with the medium of normal CE cell cultures, or of cultures of BHK21 cells of early passages. Additional of the medium of RSV-transformed CE cells or RS2 cells also increases 2- to 6-fold the residual number of foci scored 24-48 hrs after shifting from 37° to 41° CE cell cultures transformed by the ts mutant FU-19, and also about 2-fold the number of foci produced by FU-19 at 37°. This transformation-stimulating factor (TSF) reaches a plateau in the medium of producer cells 6 hrs after renewal of the medium, and is a protein, degradable by trypsin. (Contract #43219)

Foci of Rous cells produced by various RSV strains stain with methylene blue (MB) and can be distinguished from the background of nonstained density-inhibited normal CE cells. This difference is due to the fact that, whereas normal CE cells stain with MB only in log growth phase, Rous cells stain as well following confluence. Using the ts mutant FU-19, it was shown that, after shifting infected cultures from the permissive (37°) to the restrictive (41°) temperature, foci of Rous cells can lose the transformed phenotype, i.e., disappear in unstained cultures, but remain stainable for some time. Following the reverse shift (from 41° to 37°) foci can become stainable with MB before acquiring the transformed phenotype. The assay of RSV by counting foci of stainable cells (transformed and untransformed) is more reproducible and more rapid (since stained foci can be scored already 4-5 days after infection) than the usual focus assay. (Contract #43219)

TUMOR VIRUS DETECTION SEGMENT

Dr. George J. Todaro, Chief, VLLB, DCCP, Chairman
Dr. Bernard Talbot, VLLB, DCCP, Vice Chairman

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1 CP 43380)

Title: Host Restriction of Friend Leukemia Virus

Contractor's Project Director: Dr. Ruy Soeiro

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Elucidation of the molecular mechanism of restriction induced by the FV-1 gene, i.e. the inability of the N-strain of Friend Leukemia Virus to grow in Balb/c cells and of the B-strain to grow in NIH cells.

Major Findings: ^{32}P labelled Friend Leukemia Virus virions, either N or B tropic were adsorbed to permissive or restrictive cells, at a multiplicity of infection at approximately 0.2 pfu/cell. In each case, between 22-32% of input virus radioactivity was adsorbed to the cell. Within 60 min. after the period of adsorption, 8% of ^{32}P counts were associated with the cell nuclei. After 3.5 and 7.5 hrs of infection, a greater proportion of virus counts were nuclear associated, and at all times, no difference between permissive or restrictive infections could be observed in terms of nuclear associated radioactivity. Shortly after infection, nuclear associated labelled virion nucleic acid exists primarily as pure RNA when analyzed on $CsSO_4$ density gradients showing a predominant peak of ρ 1.67 (pure RNA), and a smaller peak at ρ 1.55 (putative RNA-DNA covalently linked). The latter peak is not found in pure virus, is barely detectable in cytoplasmic extracts, and represents virion RNA covalently linked to DNA. At later time points, ^{32}P labelled material appears at ρ 1.44 characteristic of essentially pure DNA. Since all these studies are performed on formaldehyde treated nucleic acid, the peaks at ρ 1.55 and 1.44 most likely represent RNA linked covalently to DNA. Chemical and enzymatic analysis reveals all the counts at ρ 1.55 to be RNA, whereas a small fraction of counts at ρ 1.44 are resistant to alkali or RNAase degradation. These resistant counts may represent either ^{32}P orthophosphate newly synthesized into DNA, or ^{32}P RNA counts transferred to DNA linked to the 5' end. Since the RNA in ρ 1.44 material is of very high molecular weight, this would appear to represent virion input nucleic acid (RNA) integrated into host cell DNA. The analysis of these RNA counts linked to DNA by RNA-DNA molecular hybridization with Friend Virus specific DNA (Reverse Transcriptase Product) showed them to be viral RNA.

These data suggest the following model: (1) That virus adsorbs to and uncoats equally well when infecting FV-1 permissive or restrictive cell types. (2) That the appearance of viral specific RNA in the infected cell nucleus, and its apparent integration with host DNA also is not affected by the FV-1 restriction gene. These data suggest that one pathway for formation

of the "pro-virus" may be the transient integration of an RNA-DNA hybrid molecule and that FV-1 restriction occurs after this event. The integration of viral RNA into host cell DNA is affected by conditions which interfere with provirus formation, such as viral infection of contact-inhibited cells, or infection in the presence of Ethidium Bromide or FuDR. These series of experiments suggest that the integration of viral RNA into host DNA is not an artifact, but rather that it is consistent with the known biology of oncornavirus replication.

Significance to Biomedical Research and the Program of the Institute:

Genetic susceptibility to cancer in humans is demonstrated by many examples. A partial listing of apparently recessive conditions includes those associated with visible chromosomal disorders. Down syndrome children have a 10-20 fold increase in incidence of leukemia. Other trisomic states (trisomy D and Kleinfelter's Syndrome) likewise have increased leukemic incidence. Xeroderma Pigmentosum, an autosomal recessive inherited syndrome, is associated with a high incidence of skin cancer. Fanconi's Syndrome (autosomal recessive) shows increased risk of leukemia in both homozygotes and heterozygotes. Familial cancers with apparent dominant gene(s) resulting in a highly penetrant genetic predisposition to cancer include the neurofibromatoses, retinoblastoma and other embryonal tumors, pheochromocytoma, and polyposis of the colon. Common cancers including breast, uterine, gastric and lung cancers all appear in increased incidence in some families.

The availability of the murine oncornavirus system, with congenic lines of mice differing in specific loci known to be associated with resistance or permissiveness to leukemia virus replication, serves as an excellent model to study genetic control of tumor virus replication.

Proposed Course: In the coming year, work will center on confirming and extending the results on the fate of input viral ribonucleic acid, as well as extending this to evaluate the synthesis of new viral DNA and proteins, in permissive and restrictive cells.

Date Contract Initiated: June 25, 1974

Current Contract Level: $31,520 (for 6 months)

BAYLOR COLLEGE OF MEDICINE (NO1 CP 53527)

Title: Nonsense Suppressor Mutants in Mammalian Cells

Contractor's Project Director: Dr. Thomas Caskey

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To develop suppressor mutants of mammalian cell lines to eventually use these to investigate the genes involved in maintaining the transformed state in cell cultures.

Major Findings: The codons UAA, and UGA have been demonstrated by the contractor to direct polypeptide chain termination in Chinese hamster, rabbit reticulocyte, and mouse extracts. Thus it can be assumed that they would be translated as premature chain termination signals when they occur within a gene as the result of a mutation.

Clones of Chinese hamster cells that are stably resistant to 8-azaguanine (8-AG^r) have been derived from V-79 under selective pressure of the toxic guanine analogue. The frequency of this occurrence has been greatly increased (>100X) over the spontaneous level when cells were treated with nitroso-guanidine (MNNG), ethyl-methane sulfonate (EMS), or ultraviolet irradiation (UV). Biochemical study of the enzyme hypoxanthine-guanine phosphoribosyl-transferase (HGPRT) isolated from such clones has assured the contractor that he has isolated clones whose resistance is related to a structurally altered HGPRT and therefore most probably the result of a mutation in the HGPRT structural gene. Some clones have a temperature sensitive HGPRT. Other clone extracts have no detectable HGPRT enzymic activity but do have proteins that react with immunoglobulin directed against HGPRT (CRM+). Approximately 40% of their clones have been found to have no detectable HGPRT by either immunologic or enzymic criteria. These clones are particularly useful for isolation of nonsense suppressors since they bear properties compatible with nonsense mutants of microbial origin.

Clones with 8-AG^r, HGPRT$^-$, and CRM$^-$ characteristics have been subjected to reversion analysis using the HAT selective system. Revertant clones arising spontaneously, and after MNNG, EMS, and ultraviolet mutagenesis have been examined. These studies identified clones which do not revert, some that revert 10^2 to 10^3 fold more frequently after MNNG and/or EMS mutagenesis, and still others that revert more frequently after UV treatment. Thus it is concluded that individual mutants can be distinguished by their reversion properties and this has reassured the contractor that, (a) a spectrum of different HGPRT$^-$ mutants have been isolated, and (b) that most (80%) can be reverted and therefore are not the result of deletion mutations. Furthermore, biochemical analysis of the clones arising in HAT media has provided evidence that some clones with HGPRT$^-$, CRM$^-$ character are the result of mutations affecting the HGPRT structural gene. This conclusion is based upon the identification of revertant clones with HGPRT which differs in its immunologic reactivity from the wild-type HGPRT.

An *in vitro* method for detecting small quantities of tRNA^{UAG} in Chinese hamster cells has been developed. This method utilizes a nonsuppressing *E. coli* extract for the translation of R17 bacteriophage RNA, with an amber mutation (sus3) in the coat gene. This extract is supplemented with Chinese hamster tRNA and aa-tRNA synthetases and is inhibited by immunoglobulin directed against release factor 1 (UAA/UAG) not RF-2 (UAA/UGA). This approach enhances the detection of suppression of the UAG mutant codon without affecting the natural coat termination signal (UAA/UAG). It is potentially useful for detection of tRNA^{UAG} or tRNA$^{UAA/UAG}$ which may be present in Chinese hamster tRNA preparations derived from the revertant clones. The method has been used to survey 20 clones.

Significance to Biomedical Research and the Program of the Institute:

Understanding of the mechanism of action of tumor viruses will require an understanding of the specific genes of the viruses that are responsible for transforming a normal cell into a tumor cell. For these studies, viral and cellular mutants will be essential. Suppressor mutants have a unique advantage over temperature sensitive mutants, in that they are "absolute, non-leaky" mutants.

Proposed Course: Contract will terminate August 15, 1975

Date Contract Initiated: January 15, 1972

Current Contract Level: $49,235

BIOLABS, INC. (NO1 CP 22068)

Title: Development and Evaluation of Methods for Large Scale Preparation of Purified Oncogenic Herpesviruses

Contractor's Project Director: Dr. Clyde R. Goodheart

Project Officer (NCI): Dr. Dharam V. Ablashi

Objectives: The development and evaluation of methods for large scale production of purified oncogenic herpesviruses (especially Herpesvirus saimiri) for biochemical, immunologic and virologic studies.

Major Findings:

Herpesvirus saimiri (HVS) was titrated simultaneously in the cell lines VERO (mycoplasma free) and VERO-N (heavily mycoplasma contaminated) in each case with standard medium (ERM) and with arginine-deficient medium (ERM-Arg). In ERM, the VERO-N gave higher plaque counts than did VERO cells. However, if arginine was omitted from the medium, equally high counts were obtained on both cell types and both were higher than with ERM. Changing the cells to ERM-Arg one or two days after infection resulted in much higher yields of virus than changing the cells immediately to deficient medium or than keeping the controls on complete ERM.

Several HVS stocks have been assayed on VERO-N, VERO and CV-1 cells using either agar or methocel as the overlay. Not only are the plaques larger with methocel, but the counts have been higher consistently by 10- to 100-fold when methocel is used as the overlay.

Recently two new lines of owl monkey kidney cells (OMK) have been tested for HVS growth. These lines are resistant to treatment with DEAE (DEAE is quite toxic to CV-1 cells) and therefore it is possible to pretreat the cells to improve the virus yield. If DEAE (25 µg/ml) is applied to the cells for 1 hour, then thoroughly rinsed off, before infection, about 100-fold higher virus yields result than when the cells are not so pretreated.

Methanol precipitation of HVS from large volumes of culture fluids was found to be useful to concentrate virus prior to further purification.

The HVS DNA that banded in CsCl at a density of 1.729 g/cm^3 was transcribed with E. coli RNA polymerase. HSV2 DNA is only 38% as efficient and HVS DNA is only about 3% as efficient as rabbit DNA in directing RNA synthesis by the complete E. coli RNA polymerase. The cRNA that is made appears to be from only a small portion of the genomes.

Significance to Biomedical Research and the Program of the Institute:

Herpesvirus saimiri induces a fatal lymphoma and/or leukemia in various primates. Mortality is almost 100% even in animals inoculated as adults; this is most unusual for any oncogenic virus. In certain aspects the disease in non-human primates induced by HVS resembles Burkitt's lymphoma in man.

Proposed Course: This contract terminated on December 19, 1974.

Date Contract Initiated: December 20, 1971

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01 CP 20208)

Title: NCI-ERDA Viral Carcinogenesis Program

Contractor's Project Director: Dr. F. T. Kenney

Project Officer (NCI): Dr. Adi Gazdar

Objectives: In January 1963 an Interagency Agreement was established between the National Cancer Institute and the Oak Ridge National Laboratory of the Atomic Energy Commission for carcinogenesis studies. In September 1972, the Viral Oncology funded portion was split out as this separate Interagency Agreement. The objective is to pursue a multidisciplinary research program on the central aspects of viral oncology.

Major Findings: Soluble extracts of mouse cells with Fv-1^n or Fv-1^b gene alleles specifically and reciprocally inhibit infection of B- or N-tropic mouse leukemia viruses in permissive cell cultures. NB-tropic virus infection was not inhibited by either cell extract. Extracts from Fv-1$^-$ cells did not inhibit infection by the three virus host-range types, but N- or B-tropic virus infection of Fv-1$^-$ cells was inhibited by extracts of the nonpermissive cells, and Fv-1^{nb} cell extracts inhibited both viruses. The inhibitor(s) is relatively stable since activity is lost after 2 hrs. at 37^o or 30 min at 56^{o}C. The inhibitor(s) was most effective if added two hours before or within two hours after infection, did not react with the virus directly, inhibit virus attachment, or inhibit the normal cell functions tested. These results indicate that nonpermissive mouse cells contain a product, possibly determined by the Fv-1 gene which inhibits some early postpenetration event(s) in leukemia virus infection. Incubation of the extracts with DNase, RNase and trypsin have shown that the inhibitory

effect is sensitive only to RNase. Further biochemical characterization of the inhibitor(s) indicates it is a macromolecule excluded by G25 Sephadex gel; its stability increases as it is purified by DEAE-cellulose and phosphocellulose chromatography; and it is present mainly in the cytoplasm of the cells (microsome fraction and post-microsomal supernatant).

The 34S RNA subunits of several oncornaviruses have been fractionated on poly(U)-Sepharose columns into those which contain poly(A), and those which do not. When [^{3}H]-labeled DNA was prepared in an endogenous reaction of viral lysates, approximately 50% of the DNA was found to hybridize with each viral RNA fraction. When the DNA fractions were reisolated, they were found to rehybridize to their original RNA complement but not the other, thus indicating that the two RNA fractions were indeed different one from the other.

Normal B6C3F$_1$ mouse serum contains antibodies specific for endogenous murine leukemia virus directed against the viral envelope proteins gp71, gp43 and p15. The antigens recognized by autogenous immune sera do not differ with respect to age of the animals, nor are they significantly different in sera from various strains of mice (BALB/c, C57BL/6, C3H/Anf). The 19S antibody fraction of B6C3F$_1$ serum reacted with gp71, gp43 and p15. In contrast, the 7S component reacted only with p15. Against BALB:virus-2, a xenotropic endogenous mouse type C virus, 19S and whole serum, but not the 7S fraction, possessed neutralizing activity.

When cellular 4S RNA is mixed with AMV or AKR viral 35S RNA at 55-60°C in a 0.1 M NaCl solution for 16 hours, specific species of 4S RNAs, demonstrated to be one prominent peak and 3-4 minor peaks by reversed phase chromatography, are found to associate with the viral RNA. After hybridization with cellular 4S RNA, the AMV 35S RNA regains its template activity with reverse transcriptase suggesting that the cellular 4S RNA provides the primer function. tRNA^{trp} constituted 50-60% and tRNA^{lys} constituted 10-20% of the major peak of chicken embryo culture cell 4S RNA which specifically become associated with the AMV 35S RNA in vitro. However, there was no evidence of the presence of tRNA^{trp} or tRNA^{lys} in the 60-70S associated 4S RNA of AKR MuLV or among the specific 4S RNA of AKR cells which hybridize in vitro to AKR-MuLV 35S RNA.

Lithium di-iodosalicylate can be used for the solubilization and purification of viral gp71; using this procedure the antigenicity of gp71 to normal mouse sera is not lost.

Experiments with cordycepin inhibition of induction of murine leukemia virus production by IdU led to the following model: Immediately after IdU incorporation and perhaps during the G-2 phase, RNA synthesis and processing (as detected by cordycepin inhibition) is required to establish in a stable form the "activation intermediate". Presumably this RNA synthesis is not related to viral structural protein synthesis as detected by immunofluorescent techniques but rather is regulatory in function. Once the intermediate is established, synthesis of virus protein proceeds after DNA synthesis and cell division.

λ-radiation delivered at a dose rate of ∿20 R/hr can reproducibly activate virus in AKR cells. Thus far the minimum total dose which activates virus is ∿350 R, when delivered to actively dividing cells. If cell division is repressed by serum deprivation, virus activation is significantly reduced.

Most cellular DNA polymerases from several sources (e.g., nonactivated AKR cells, normal mouse spleen) have no affinity for poly(Um)-Sepharose columns at low salt concentrations. Reverse transcriptase (from RLV, AKR virus, activated AKR cells, or RLV-infected spleens) binds to the column and can be eluted with an NaCl gradient, leading to separation of the viral enzyme from most cellular DNA polymerases.

Pretreatment with poly(Am) significantly inhibits the induction of sarcomas by Moloney sarcoma virus in newborn mice. Treatment of the mice four hours before MSV inoculation was much more effective than one hour pretreatment. Inoculation of the drug at the time of virus infection, even when subsequent doses were given after infection, was not effective. High virus concentrations could overcome the inhibitory effect of poly(Am). The effects of poly(Am) on RLV infection were tested in young adult BALB/c mice. When poly(Am) was given one hour before virus infection there was an enhanced degree of splenomegaly, but when given four hours before virus there was a slight, but not significant, inhibition of splenomegaly.

Significance to Biomedical Research and the Program of the Institute:

This effort is focused on the phenomenon of viral carcinogenesis; the problem is investigated in terms of cell biology, enzymology, and control of gene expression. This multifaceted approach studies primarily the mouse leukemia system, but increasingly the findings are being carried over into work with human tumor cells in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: The study of Immunology of Viral Carcinogenesis will be terminated. Work will continue on Regulation of Gene Expression (Kenney), RNA Tumor Virus Cell Biology (Tennant) and Enzymology of Viral Carcinogenesis (Yang).

Date Contract Initiated: July 1, 1963 (Separate Viral Oncology Contract: September 1, 1972)

Current Contract Level: $561,374

FONDATION CURIE-INSTITUT DU RADIUM (NO1 CP 43219)

Title: Molecular and Genetic Studies of Rous Sarcoma Virus

Contractor's Project Director: Dr. R. Latarjet

Project Officer (NCI): Dr. Robert Bassin

effect is sensitive only to RNase. Further biochemical characterization of the inhibitor(s) indicates it is a macromolecule excluded by G25 Sephadex gel; its stability increases as it is purified by DEAE-cellulose and phosphocellulose chromatography; and it is present mainly in the cytoplasm of the cells (microsome fraction and post-microsomal supernatant).

The 34S RNA subunits of several oncornaviruses have been fractionated on poly(U)-Sepharose columns into those which contain poly(A), and those which do not. When [^{3}H]-labeled DNA was prepared in an endogenous reaction of viral lysates, approximately 50% of the DNA was found to hybridize with each viral RNA fraction. When the DNA fractions were reisolated, they were found to rehybridize to their original RNA complement but not the other, thus indicating that the two RNA fractions were indeed different one from the other.

Normal B6C3F$_1$ mouse serum contains antibodies specific for endogenous murine leukemia virus directed against the viral envelope proteins gp71, gp43 and p15. The antigens recognized by autogenous immune sera do not differ with respect to age of the animals, nor are they significantly different in sera from various strains of mice (BALB/c, C57BL/6, C3H/Anf). The 19S antibody fraction of B6C3F$_1$ serum reacted with gp71, gp43 and p15. In contrast, the 7S component reacted only with p15. Against BALB:virus-2, a xenotropic endogenous mouse type C virus, 19S and whole serum, but not the 7S fraction, possessed neutralizing activity.

When cellular 4S RNA is mixed with AMV or AKR viral 35S RNA at 55-60°C in a 0.1 M NaCl solution for 16 hours, specific species of 4S RNAs, demonstrated to be one prominent peak and 3-4 minor peaks by reversed phase chromatography, are found to associate with the viral RNA. After hybridization with cellular 4S RNA, the AMV 35S RNA regains its template activity with reverse transcriptase suggesting that the cellular 4S RNA provides the primer function. tRNA^{trp} constituted 50-60% and tRNA^{lys} constituted 10-20% of the major peak of chicken embryo culture cell 4S RNA which specifically become associated with the AMV 35S RNA in vitro. However, there was no evidence of the presence of tRNA^{trp} or tRNA^{lys} in the 60-70S associated 4S RNA of AKR MuLV or among the specific 4S RNA of AKR cells which hybridize in vitro to AKR-MuLV 35S RNA.

Lithium di-iodosalicylate can be used for the solubilization and purification of viral gp71; using this procedure the antigenicity of gp71 to normal mouse sera is not lost.

Experiments with cordycepin inhibition of induction of murine leukemia virus production by IdU led to the following model: Immediately after IdU incorporation and perhaps during the G-2 phase, RNA synthesis and processing (as detected by cordycepin inhibition) is required to establish in a stable form the "activation intermediate". Presumably this RNA synthesis is not related to viral structural protein synthesis as detected by immunofluorescent techniques but rather is regulatory in function. Once the intermediate is established, synthesis of virus protein proceeds after DNA synthesis and cell division.

λ-radiation delivered at a dose rate of ∿20 R/hr can reproducibly activate virus in AKR cells. Thus far the minimum total dose which activates virus is ∿350 R, when delivered to actively dividing cells. If cell division is repressed by serum deprivation, virus activation is significantly reduced.

Most cellular DNA polymerases from several sources (e.g., nonactivated AKR cells, normal mouse spleen) have no affinity for poly(Um)-Sepharose columns at low salt concentrations. Reverse transcriptase (from RLV, AKR virus, activated AKR cells, or RLV-infected spleens) binds to the column and can be eluted with an NaCl gradient, leading to separation of the viral enzyme from most cellular DNA polymerases.

Pretreatment with poly(Am) significantly inhibits the induction of sarcomas by Moloney sarcoma virus in newborn mice. Treatment of the mice four hours before MSV inoculation was much more effective than one hour pretreatment. Inoculation of the drug at the time of virus infection, even when subsequent doses were given after infection, was not effective. High virus concentrations could overcome the inhibitory effect of poly(Am). The effects of poly(Am) on RLV infection were tested in young adult BALB/c mice. When poly(Am) was given one hour before virus infection there was an enhanced degree of splenomegaly, but when given four hours before virus there was a slight, but not significant, inhibition of splenomegaly.

Significance to Biomedical Research and the Program of the Institute:

This effort is focused on the phenomenon of viral carcinogenesis; the problem is investigated in terms of cell biology, enzymology, and control of gene expression. This multifaceted approach studies primarily the mouse leukemia system, but increasingly the findings are being carried over into work with human tumor cells in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: The study of Immunology of Viral Carcinogenesis will be terminated. Work will continue on Regulation of Gene Expression (Kenney), RNA Tumor Virus Cell Biology (Tennant) and Enzymology of Viral Carcinogenesis (Yang).

Date Contract Initiated: July 1, 1963 (Separate Viral Oncology Contract: September 1, 1972)

Current Contract Level: $561,374

FONDATION CURIE-INSTITUT DU RADIUM (NO1 CP 43219)

Title: Molecular and Genetic Studies of Rous Sarcoma Virus

Contractor's Project Director: Dr. R. Latarjet

Project Officer (NCI): Dr. Robert Bassin

Objectives: To study Rous Sarcoma Virus including the phenomena of infectious DNA, viral antigens and temperature sensitive viral mutants.

Major Findings: Infectious DNA able to elicit the production of virus in permissive chick embryo cells (CE cells) can be recovered from transformed CE cells producing either RS-RSV (D) or the ts mutant FU-19 of this virus, and also from nontransformed CE cells producing either RAV 1 or RAV 50. DNA is infectious only when cells are pretreated with DEAE-dextran; the optimal dose is about 100 µg/ml, for 30-60 min before infection with DNA.

Cultures of CE cells infected with Bryan strain RSV (B-RSV(RAVI)) in the presence of the medium of CE cell cultures transformed by SR-RSV, or of RS2 cell cultures, can increase 2- to 6-fold the number of foci of Rous cells, relative to untreated controls, or cultures incubated with the medium of normal CE cell cultures, or of cultures of BHK21 cells of early passages. Addition of the medium of RSV-transformed CE cells or RS2 cells also increases 2- to 6-fold the residual number of foci scored 24-48 hrs after shifting from 37° to 41° CE cell cultures transformed by the ts mutant FU-19, and also about 2-fold the number of foci produced by FU-19 at 37°. This transformation-stimulating factor (TSF) reaches a plateau in the medium of producer cells 6 hrs after renewal of this medium, and is a protein, degradable by trypsin.

RSV of A, B, C or D subgroups (types) is inactivated by incubation with antiserum against normal chick embryo (CE) cells, in the presence, but not in the absence, of complement. This inactivation is not due to Forssman antigen, nor to the action of antiserum and complement on the CE cell cultures used for the assay of the inactivated RSV. However, since gs^+ h^+ CE cells can carry on their surface the gp85 envelope antigen of their cryptic type C virus (chf), and since CE cells used for preparing the antiserum (in rabbits) were gs^+, the surface antigen common to RSV and CE cells could be gp85 of chf. This was tested by absorbing the anti-CE serum with gs^- CE cells which do not carry gp85 of chf. The absorption removed the inactivating capacity of the serum. Hence, the antigen common to RSV and CE cells is presumably a normal cell-coded antigen. Parallel electron microscopy studies have shown that virions treated with anti-CE serum and complement, but not antiserum or complement only, become swollen, loose their opacity to electrons, and are also less numerous than in control preparations. Therefore, the antiserum and complement treatment could be virolytic. This was checked by showing that, following treatment with antiserum and complement, the viral 70S RNA becomes digestible by RNAse, and the gs antigen is released from the particles. RSV produced by heterokaryons formed by fusion of RSV-transformed virogenic cells (RS2 clone of hamster BHK21 cells transformed by SR-RSV-D) and CE cells also carries the HSCA common to CE cells and RSV produced by these cells, but no antigen of the virogenic cell. Hence, RSV which is produced in heterokaryons matures only at chicken-specific sites of their surface, which suggests that the chicken HSCA may be required for virus maturation.

Indirect immunofluorescence tests with serum of guinea pigs hyperimmunized with SDS-dissociated RSV showed the presence of gs antigen in acetone-fixed

preparations of all RSV transformed mammalian cell lines studied. The highest titers of antigen were in the 17 RBI cells and in XC, RBH, RSH, RS2/3 and RS2/10 cells, i.e., in the permissive and the most inducible nonpermissive cells. The lowest titers were in the TWERC and in the RS2/6 and RS2/7 cells, i.e., in the non-inducible or least-inducible cells.

The capacity of hamster BHK21/13 cells to form colonies in suspension in agarose medium is reduced markedly by preincubation with normal guinea pig (GP) serum, whereas the colony forming capacity of GHK21/13 cells transformed by SR-RSV (RS2 cells) is much less affected by this treatment. This suggests that the expression of a hamster-specific antigen (HSA) detected by GP serum, may be markedly reduced following transformation by RSV.

Foci of Rous cells produced by various RSV strains stain with methylene blue (MB) and can be distinguished from the background of nonstained density-inhibited normal CE cells. This difference is due to the fact that, whereas normal CE cells stain with MB only in log growth phase, Rous cells stain as well following confluence. Using the ts mutant FU-19, it was shown that, after shifting infected cultures from the permissive (37°) to the restrictive (41°) temperature, foci of Rous cells can lose the transformed phenotype, i.e., disappear in unstained cultures, but remain stainable for some time. Following the reverse shift (from 41° to 37°) foci can become stainable with MB before acquiring the transformed phenotype. The assay of RSV by counting foci of stainable cells (transformed and untransformed) is more reproducible and more rapid (since stained foci can be scored already 4-5 days after infection) than the usual focus assay.

Studies of BHK21/13 cells transformed by SR-RSV-D (RS2 cells) showed that the most inducible clones of transformed cells, which are also the richest in viral gs antigen and in VISA, regularly grow to higher cell densities than untransformed BHK cells or cells of the least inducible clones. The main difference between untransformed and transformed cells appears to be that the latter cells are able to use more efficiently all the components of the medium. Renewing of the medium of confluent cultures arrested in their growth by lack of medium renewal, results in an earlier resumption of growth of the transformed cells. Hence, these cells appear to have a shorter G1 phase, or to be arrested closer to the S phase than untransformed cells.

Infection of neuroretinal (NR) cells from 7 day-old chick embryos (CE) with wild type SR-RSV results in massive morphological transformation accompanied by viral replication. Morphological transformation was evident in most cells within 36 hrs. after infection and differed from that observed in CE fibroblasts, in that transformed NR cells were initially considerably enlarged, polygonal, or elongated with abundant cytoplasm and numerous extensions. These cells started to multiply and, by day 3 or 4, gave rise to typical Rous cells which proliferated in multilayers while constantly releasing virus, and could be subcultured eight times, over a 2 month period.

CE fibroblasts, were infected with 0.1 to 1 FFU/cell of SR-RSV(D) and one of the following inhibitors: Arabinoside cytosine, Actinomycin D, Cycloheximide. The three inhibitors had the same effect; a significant decrease of

the virus titer was observed as early as 45 min after the beginning of the infection period, showing that the duration of the latent period of RSV can be as short as 45 min.

Significance to Biomedical Research and the Program of the Institute:

This work will aid in the goal of determining the relationship of viruses to the etiology of human cancer. Because of the profound similarities between oncornaviruses, Rous Sarcoma Virus (RSV) is an excellent model system for the study of these viruses, including those which can be expected to be found in human tumors.

Proposed Course: Contract will terminate October 14, 1975

Date Contract Initiated: October 15, 1973

Current Contract Level: $52,000

HARVARD UNIVERSITY (NO1 CP 33390)

Title: Oncogenic Herpesviruses in Primates

Contractor's Project Director: Dr. T. C. Jones

Project Officer (NCI): Dr. Gary Pearson

Ojbectives: To study oncogenic herpesviruses in primates especially Herpesvirus saimiri and Herpesvirus ateles.

Major Findings: Herpesvirus saimiri has been shown to induce malignant lymphoma in New Zealand white rabbits. Rabbits inoculated by the intravenous route developed malignant lymphoma within 17 to 196 days. A single inoculation (2 ml for 4 month old rabbits, 1 ml for weaned and 0.5 for baby rabbits) induced lymphomas in a majority of the rabbits. A unique feature observed in the rabbit lymphomas is the invasion of the skin of the eyelid and nose with lymphocytes or lymphoblasts. This feature has not been reported in studies involving non-human primates injected with H. saimiri. In rabbits that died of lymphoma after 46 days, there was severe conjunctivitis, nasal discharge and dyspnea. This clinical picture has also not been observed in monkeys developing malignant lymphoma. Baby rabbits have developed lymphocytic leukemia. This was not a consistent feature in older rabbits with the exception of one rabbit which developed a terminal leukemia.

DFL-H. saimiri has been passaged in dog fetal lung cell cultures for more than 30 times. The titer of this virus increased in DFL from a titer of 0.0/ml to 4.5/ml after 32 passages and decreased from 5.5/ml to 1.0/ml in owl monkey kidney cell cultures. The DFL-H. saimiri was neutralized by and fluoresced specifically with H. saimiri antisera. The 5th, 22nd, and 31st passages have been tested in 3 groups of owl monkeys. These passages did not protect the animals when challenged with the parent virus. However, the animals which succumbed to disease after challenge showed a less invasive disease as determined by histopathological studies.

Two owl monkeys were placed in adjoining cages with two H. saimiri infected squirrel monkeys. The 2 squirrel monkeys were excreting virus by the oropharyngeal route beginning 15 days after virus inoculation and virus was also recovered from peripheral blood. The inoculated squirrel monkeys did not develop any disease during the period of observation which lasted over 1 year. One of the owl monkeys after 3 months of contact was positive for H. saimiri. Virus was consistently recovered from the peripheral blood. The presence of virus was detected for 9 months. This animal died after 11 months of uremia. There was no evidence of malignant lymphoma. H. saimiri was recovered from kidney cell cultures of this animal. The second owl monkey is still alive and appears to be normal.

H. saimiri was able to infect human cell cultures (whole human embryo and human embryonic cell cultures). Although the virus multiplied in these cell cultures, it failed to induce a complete cytolytic infection as observed in owl monkey kidney cells. Virus could be recovered from these infected human cells up to 187 days. After this period no virus could be detected, but by the cell fusion technique virus was recovered at the end of 347 days.

Significance to Biomedical Research and the Program of the Institute:

The knowledge gained in the study of these herpesviruses will provide insight into malignant processes of man in which herpesviruses are considered likely candidates for etiology: Epstein-Barr virus (EBV) in Burkitt's lymphoma, and Herpes simplex type 2 in carcinoma of the cervix, and will provide well characterized antigens and other reagents for testing for presence of related viruses in humans.

Proposed Course: Work will continue on 1) Karyotypic studies of owl monkeys, and analysis of the differing response of different Karyotypes to H. saimiri; 2) susceptibility of rabbits to H. saimiri; 3) natural horizontal transmission of H. saimiri; and 4) testing the oncogenicity of H. ateles and new primate herpesviruses in various primates.

Date Contract Initiated: June 26, 1972

Current Contract Level: $96,000

HARVARD UNIVERSITY (NO1 CP 43299)

Title: Investigation of Cellular, Exogenous and Endogenous RNA Viral Factors in Replication and Transformation of Polyoma Virus.

Contractor's Project Director: Dr. Thomas L. Benjamin

Project Officer (NCI): Dr. Brenda Gerwin

Objectives: To investigate the relationship between cells transformed by DNA and RNA tumor viruses. Specifically to test the ability of various cell lines, transformed or infected by various DNA or RNA tumor viruses, to complement the growth defect of the polyoma virus mutant, NG-18.

Major Findings: Mutants of polyoma have been isolated which fail to make plaques on the 3T3 line of mouse embryo fibroblasts while retaining the ability to make plaques on a polyoma transformed derivative of 3T3. A general characteristic of this class of host range mutants is their inability to cause cell transformation in vitro. NG-18, a prototype of the non-transforming host range mutants, is also defective in its ability to cause tumors following injection into newborn hamsters. The host range and non-transforming properties are closely linked, since all of eleven mutants isolated thus far are defective in transformation of rat and hamster fibroblasts; in addition, lack of complementation among these mutants indicates that a single viral gene is involved. NG-18, screened originally for its ability to grow in polyoma-transformed cells, has now been shown to grow in a variety of other cell types. Among cells which are substantially permissive for the growth of NG-18 are: phenotypic revertants of polyoma-transformed cells, type C RNA virus transformed and/or producing cells, cells which become transformed upon infection with leukemia virus, primary baby mouse kidney epithelial cells, and primary mouse embryo fibroblasts. Among cells which are poor hosts for the growth of NG-18 are: all 3T3 cell lines, SV-40 transformed 3T3 cells, spontaneously transformed 3T3 cells, radiation and chemical carcinogen transformed 3T3 cells, and late passage mouse embryo fibroblasts. Similar results have been obtained using three other independently isolated host range mutants of the same type as NG-18. The permissive state thus does not require a cell-associated polyoma genome as the basis for complementation of growth for this class of host range mutant. In addition, these results demonstrate that cells can be permissive for the growth of such non-transforming virus mutants without themselves possessing properties commonly associated with transformation: loss of density dependent inhibition of growth, low serum requirement for growth, loss of anchorage dependence of growth, and lectin agglutinability.

Significance to Biomedical Research and the Program of the Institute:

The study deals with extending and confirming earlier results which seem to indicate common cellular functions expressed following transformation by either RNA or DNA viruses. This is important since it may help to zero in on the "essense" of transformation. Ultimately this could lead to the identification and eventual isolation of a hypothetical "transforming protein", which would then open the way for design of specific drugs for control or prevention of cancer.

This study involves the use of host range mutants of tumor viruses. Many NCI contracts and grants are investigating temperature-sensitive mutants of tumor viruses. The advantages of host range mutant studies over temperature sensitive mutant studies are: (1) Host range mutants may in principle be of any structural type, and do not depend on a "quasi-" or conditionally functional gene product. Missing subunits, deletions of portions of subunits, frame-shifts, inversions, etc. are all possible bases of host range mutants which would depend not on their own product for growth under permissive conditions but on being complemented by homologous cell-associated viral functions. (2) The inherent leakiness of temperature-sensitive mutants can be and often is a severe drawback, risking high background levels of gene functions at non-permissive temperature, which affects both complementation

and physiological experiments. (3) Since non-permissive temperature is usually several degrees above normal body temperature for the murine system, it can be expected that the inherent leakiness would be an even greater problem in in vivo experiments. Thus, ts mutants are not ones of choice for attacking the important question of pathogenic properties of altered viruses. (4) Temperature sensitive mutants are typically of the mis-sense type, usually stemming from a single base change; thus, they are subject to reversion and intra-genic complementation. (5) Viral-directed protein synthesis in in vitro systems is just beginning to be approached with tumor viruses; though a technically difficult task, this may be viewed as the ultimate way of identifying and characterizing viral gene products. In this regard, host range mutants with drastic alterations in the RNA would be necessary; ts mutants which code for products chemically similar to the wild type would be difficult to use in this approach.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: April 1, 1974

Current Contract Level: $66,500 (for 6 months)

HEBREW UNIVERSITY (NO1 CP 33310)

Title: Studies on Herpesvirus (EBV) and its Role in Human Cancer

Contractor's Project Director: Dr. Yechiel Becker

Project Officer (NCI): Dr. Berge Hampar

Objectives: The work concentrates on four main areas: (1) The composition and internal organization of EBV-DNA. The molecular composition and properties of EBV-DNA will be investigated (the presence of alkali sensitive bonds, denaturation properties, G + C rich regions in the DNA, electrophoresis in acrylamide gels). (2) The state of EBV-DNA in Burkitt lymphoblasts. The relationship between EBV-DNA and the host DNA genome will be studied utilizing electrophoresis in acrylamide gels to separate viral DNA from host cell DNA. The nature of the cellular DNA synthesized in Burkitt lymphoblasts after arginine deprivation, at the time of EBV-DNA replication, will be studied. (3) Transcription of EBV-DNA in the lymphoblasts. The nature of EBV mRNA synthesized in Burkitt lymphoblasts will be studied using cells prior and after induction of virus synthesis with BrdU or arginine deprivation. Poly-A-containing mRNA species will be isolated and their specificity studied by hybridization to EBV-DNA. (4) Translation of EBV genetic information in Burkitt lymphoblasts. The viral structural peptides synthesized in virus producing and nonproducing cell lines and their intracellular localization will be investigated. Virus specific surface antigens will be characterized.

Major Findings: Experiments were carried out on EBV DNA containing Raji cells, which cannot be stimulated to produce EB virus by all known techniques. The results showed that the EBV DNA sequences banded in the

position of cell DNA (density 1.70 g/ml). EBV DNA was also detected at the density of 1.707 g/ml when a sheared DNA preparation was used. Similarly, alkali denaturation resulted in the appearance of EBV DNA sequences in the position between the cellular and viral DNAs. Based on these findings it was suggested that EBV DNA might be present as fragmented molecules integrated into Raji cellular DNA.

Distamycin A inhibits the translation of some EBV genetic information in Burkitt lymphoblasts. The greatest inhibitory effect occurred with the synthesis of peptide number II (the capsid protein) and peptides III, IV and V (the glycoproteins). In P_3HR-1 cells, distamycin A prevented the appearance of viral capsid antigen (VCA) while early antigen (EA) was not affected. These results indicate that distamycin A, which binds to A-T sequences in the DNA and inhibits transcription by DNA and RNA polymerases, selectively inhibits the transcription of the mRNA which contains the information for VCA (=peptide II). In this respect EBV DNA differs from HSV DNA since distamycin A does not prevent the synthesis of capsid peptide II in HSV infected cells.

The EBV nuclear antigen described by Klein (EBNA) was extracted from Raji cells by 0.03 M sodium deoxycholate or 1.0 M sodium chloride. Four peptides were found in the 1.0 M NaCl extract which had molecular weights of 55,000, 45,000, 30,000 and 4,000. The 0.03 M sodium deoxycholate extracted eight peptides. It is not yet possible to conclude which peptide is EBNA.

HSV does not reproduce in XC cells (rat cells transformed in vitro by Rous sarcoma virus (RSV)). However, it does reproduce in normal rat cells or in the RB 77 cell-line (another cell-line which resulted from the transformation of rat cells by RSV).

Previous studies on the isolation of EBV from Burkitt lymphoblasts were done with the EB_3 strain which was induced by incubating the cells in an arginine deficient medium or in a complete medium at 33°C. A new batch of EB_3 cells were now found to be resistant to induction with either iododeoxyuridine (IUdR), incubation of the cells at 33°C or incubation in an arginine deficient medium. These experiments demonstrated that the continuous cultivation of Burkitt lymphoblasts led to the selection of cells which contain the EBV DNA genomes in a form that cannot be induced by the techniques now available.

P_3HR-1 Burkitt lymphoblasts and Raji cell lines of Burkitt lymphoblasts as well an another human lymphoid cell line (1301) which does not contain EBV DNA were infected with herpes simplex virus type 1 (strain HF). Infectious virus was synthesized in each of the three cell-lines as determined by plaque assays on BSC_1 (green monkey) monolayers.

Significance to Biomedical Research and the Program of the Institute:

EBV appears to be associated with Burkitt's lymphoma. Since the members of the herpesvirus group are usually viruses which cause lytic infections in sensitive cells, the non-lytic relationship between EBV and its host, the

lymphoblast, represents an unusual type of cell-virus interaction. The Burkitt lymphoblasts in human tumors contain EBV-DNA in a repressed state. Only when the cellular mechanisms are changed, expression of the viral genes takes place and EB virions are synthesized. The nature of the mechanisms in the lymphoblasts that control viral gene functions are not yet known. These studies will shed light on the nature of the association of EBV-DNA with the lymphoblast's genome. The studies on the molecular processes involved in the regulation of the Herpesvirus DNA genomes in permissive and transformed cells will provide basic information on the nature of the EB virus induced antigens and their role in the ability of the tumorigenic cells to proliferate and develop tumors in humans. These studies may help to assess the usefulness of these viral antigens for immunodiagnosis and immunotherapy of cancer in humans.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: June 29, 1973

Current Contract Level: $103,567

JOHNS HOPKINS UNIVERSITY (NO1 CP 43266)

Title: Studies of New Papovaviruses Isolated from Man

Contractor's Project Director: Dr. Richard T. Johnson

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Study papovaviruses recently isolated from man. (1) Attempts to isolate new papovaviruses, to characterize them, and to study if viral antigens are present in human cerebral neoplasms. (2) Epidemiological studies of the presence of these viruses and their antigens in human sera and urine.

Major Findings: Studies of SV40-PML induced cerebral neoplasms have been carried out in hamsters showing that cryostat-cut sections of tumors seldom demonstrate T antigen by fluorescent antibody staining but that cells cultured from these tumors uniformly expressed T antigen. In view of this finding, the preliminary studies of human brain tumors involved not only examination of cryostat-cut sections but cells were grown in culture and stained in sub-confluent and confluent cultures for possible T antigen expression. A series of human brain tumors examined in this way failed to demonstrate T antigen.

Biological properties of a strain of cells derived from a brain biopsy of a patient with progressive multifocal leukoencephalopathy were investigated. The patient had a high antibody titer to SV40, and SV40 was isolated from the brain cells. The brain cells were epithelioid in shape. They grew into contact inhibited monolayers and could be subcultured approximately 30 times. Thirty percent of the cells at the 11th passage were diploid and the remainder aneuploid. Although SV40 could be readily rescued from the cells,

expression of SV40 T antigen was restricted to the growth phase of the cells and was not detectable in confluent monolayers. Cells did not have V antigen by immunofluorescence, but small amounts of free virus were recovered from sonicated cell preparations. High and low molecular weight DNA extracted from the cells were infectious but most of the infectivity was associated with the high molecular weight fraction.

Urine samples were studied from cancerous patients on immunosuppressive therapy and from normal controls. The study population consisted of: 1) 23 children with leukemia or solid tumors, most of them in remission, undergoing chemotherapy as outpatients; 2) 9 cancerous adults who were hospitalized and heavily immunosuppressed; 3) age-matched normal children and adults. The frequency of BK virus-specific antibodies was significantly higher in the urines of cancer patients both by the HAI and FA-IgG tests. Aggregates of papovavirus particles were found in 6 of 41 urines from cancerous patients and in none of 21 normal urines. The 6 positive urines came from three patients - 2 adults and one child. Two out of 3 urines of patient #1, all 3 of patient #2, and 1 out of 2 of patient #3 had particles. The duration of excretion (a minimum estimate) was two weeks in patient #1 and three and a half weeks for patient #2. Patient #2 excreted very large numbers of particles. All urine samples of patient #2 and at least one of the other two had BKV-specific antibodies.

Significance to Biomedical Research and the Program of the Institute:

Papovaviruses are known to cause tumors in animals and to transform cells in culture. Since there is serological evidence that human infection by certain papovaviruses is rather common, it is important to assess the role of these viruses in human cancer.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: February 1, 1974

Current Contract Level: $69,919

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1 CP 33348)

Title: Studies on the Leukemia Virus DNA Polymerase and Terminal Deoxynucleotidyl Transferase.

Contractor's Project Director: Dr. David Baltimore

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Characterization of DNA polymerases associated with oncornaviruses and normal and neoplastic cells as well terminal deoxynucleotidyl transferase; to investigate their function, mechanisms, and products.

Major Findings: Reverse transcriptase isolated from avian RNA tumor viruses consists of two poly-peptide chains: an α subunit of 60,000 molecular weight

and a β subunit of 100,000 molecular weight. It appears that the β subunit plays a role in the binding of the holoenzyme (α,β) to template primer.

When crude extracts of secondary chick embryo fibroblasts infected and transformed by RSV were fractionated, all of the reverse transcriptase activity recoverable from phosphocellulose columns was found in the membranous fraction of the cell, and was further characterized as associated with the plasma membrane suggesting that most of the "intracellular" active enzyme could be in budding virus particles or at least in a state preparatory to budding. The reverse transcriptase activity could not be washed from the membrane fraction by high salt buffer. Furthermore, to assay the reverse transcriptase activity in the membrane fraction required addition of a nonionic detergent (nonidet P40) to break the membrane structure. These results suggest that the activity is found inside of particles.

A radioimmunoassay (R.I.A.) for reverse transcriptase was developed by purifying AMV reverse transcriptase to apparent homogeneity. Both AMV or RSV gave similar curves except that the enzyme in RSV virions was unable to displace all of the ^{125}I AMV reverse transcriptase. It is concluded that the enzymes from AMV or RSV are very similar but they have some type-specific antigenic determinants. Reverse transcriptase from murine leukemia virus (Moloney) has no common antigenic determinants with AMV. 2.5% of the proteins in the AMV virion are reverse transcriptase; this amounts to about 70 molecules of enzyme per virion.

Studies on terminal deoxynucleotidyl transferase (TdT) in humans have shown that the enzyme is an extremely good marker for cells from acute lymphoblastic leukemia. Normal people have the enzyme only in their thymocytes and, at low levels, in the bone marrow. Three forms of the enzyme have been identified: two forms which are both in thymocytes as well as acute lymphoblastic leukemia cells and one form which has thus far been found only in the bone marrow.

Multiple forms of murine TdT have also been analyzed and the distribution of the enzyme among the various types of murine thymocytes has been characterized. Most of the enzyme is sensitive to cortisone treatment and those thymocytes resisting cortisone treatment have largely that form of TdT which is ordinarily less than 10% of the total. The distribution of the two terminal transferase activities is a valuable parameter in distinguishing thymocyte subpopulations, and perhaps in establishing a developmental sequence within the thymus. Thus far all TL (Thymus Leukemia) antigen positive tumor lines carry terminal transferase activity and no TL negative line yet has been shown to have the enzyme.

Significance to Biomedical Research and the Program of the Institute:

The characterization of reverse transcriptase (the enzyme that produces DNA from the RNA of type C viruses) may provide much more sensitive techniques for locating cancer virus genetic information in human tissues. Studies of terminal deoxynucleotidyl transferase (TdT) may provide an excellent marker for those cases of chronic myelogeneous leukemia which have a lymphoblastic cell crisis.

Proposed Course: In the coming year work on reverse transcriptase will continue on the in vitro behavior of the enzyme, but more effort will be placed on defining the intracellular state of the enzyme. Both avian and murine systems will be under investigation. The second category of work involves studies on the enzyme terminal deoxynucleotidyl transferase. This enzyme is being studied as a possible diagnostic signal which can be used to classify and follow the course of treatment of certain types of human leukemia. The enzyme is a unique type of DNA polymerase apparently limited to certain lymphocyte precursor populations in normal animals.

Date Contract Initiated: May 1, 1971

Current Contract Level: $142,055

SIDNEY FARBER CANCER CENTER (NO1 CP 43269)

Title: Attempt to Isolate Type C Virus from Cultured Human Leukemia Cells

Contractor's Project Director: Dr. George E. Foley

Project Officer (NCI): Dr. Brenda Gerwin

Objectives: To attempt to isolate an intact type C virus from freshly cultured human leukemia cells, either by finding such cells which spontaneously produce virus, or by inducing virus production by physical, chemical or immunological means.

Major Findings: During the first 8 months of the contract over 250 cultures were initiated from 82 donors, derived mainly from the peripheral blood or bone marrow of patients with a variety of chronic and acute leukemia. Ten cell lines, 8 from different donors, and 2 from a common donor have been firmly established. All lines having doubling times of 18 - 28 hrs. Each line is subjected to an immunologic screen to assess whether it falls into the category of an already well-characterized human immunocompetent cell, and several lines have been analyzed by histochemical methods for three enzymes - peroxidase, esterase, and leukocyte alkaline phosphatase as well as for glycogen. Positivity of the enzyme tests and negativity of the PAS (glycogen) reaction have, in the past, served as markers of the myeloid (as opposed to lymphoid) nature of blastoid cells.

Attempts are continuing, directed at isolating an infectious, non-contaminating, type C virus from human cells. Supernatants of continuous leukemic lines are assayed in a polymerase test using the synthetic template poly rA. oligo dT in the presence of Mn^{2+}, and then each line is cocultivated with xeno- and homogeneic cell groups - for 5-6 weeks, with weekly checks on supernatants for polymerase. None of these continuous leukemia lines have been reproducibly high-level polymerase particle producers. One line, WPM-1, obtained from a patient with lymphoma has regularly yielded activities of 2-7,000 CPM/0.01 ml (40,000 CPM/pmole) over background and is now being cocultivated with xeno- and homogeneic recipients. It is also under study by electron microscopy and will be tested by radioimmunoassay for several

type C viral p30 molecules. In addition, chemical induction of potential donor cell lines with halogenated pyrimidines has begun as has cocultivation of allogeneic mixed lymphocyte cultures from normal donors and healthy individual - leukemic patient pairs. In several instances primary cultures were distinctly polymerase positive early in the course of cultivation but when continuous lines emerged in each instance, the polymerase levels fell to background.

Continuous lines of lymphoid cells taken from patients with leukemia were, unlike lines from healthy donors, unable to grow in the presence of cystathionine, and had markedly reduced activity of the enzyme, cystathionase, which cleaves cystathionine to α-ketobutyrate and cysteine. For several murine and rat lymphoma lines tested, this seemed also to be true. An improved assay for cystathionase was developed, and the use of this as an indicator of transformation of a hematopoietic cell is being investigated.

In addition to the previously described type C viral pseudotypes of Vesicular Stomatitis (VSV), the reverse now appears to have been obtained, i.e., a virus bearing KiMSV RNA and at least some VSV coat proteins.

BIOA mice appear to have as endogenous type C viruses, a xenotropic virus, an N-tropic virus, a B-tropic virus and an NB-tropic virus.

Significance to Biomedical Research and the Program of the Institute:

The successful isolation of a human type C virus would permit the development of reagents to determine if they are natural etiological agents of human cancer. For example, the ^{3}H-DNA product of the endogenous reverse transcriptase reaction of a human type C virus would be the ideal probe for the investigation of the subinfectious genetic expression of such viruses in normal and malignant human cells. The antibody to the reverse transcriptase and gs antigen and other viral specific proteins of such a particle would be superb tools for the development of analytical systems designed to detect type C viral proteins in non-productive human cells. Once one could specifically identify human viral RNA and viral proteins being synthesized in cells, a multitude of experiments, directed at delineating the role of type C viruses in natural human oncogenesis, could be performed. The biology of these viruses could be studied as well, with a view towards assessing their oncogenic potential prospectively. For these purposes, focus formation on appropriate cultured cell lines would be sought. If absent, injection of virus into animals would be attempted, under a variety of circumstances in the hopes of eliciting a tumor response. Finally, the identification of a viral specific transformation protein in transformed but not normal cells would be the strongest evidence that endogenous type C virus is really involved in the etiology and pathogenesis of human neoplasia.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: April 24, 1974

Current Contract Level: $135,508

UNIVERSITY OF CALIFORNIA (NO1 CP 33332)

Title: Hormonal Control of Gene Expression in Tumor Viruses

Contractor's Project Director: Dr. Gordon M. Tomkins

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To study (1) the mechanisms by which viral transformation disrupts cellular growth regulation, and (2) cellular mechanisms which control the expression of viral genes.

Major Findings: Glucocorticoids increase both murine mammary tumor virus (MTV) virion, and viral RNA production in cultured GR cells. GR cell glucocorticoid receptors are indistinguishable from those of S49 cells based on their sedimentation and DNA-binding properties. S49 cells contain MTV RNA in quite significant quantities. Nevertheless, S49 cells cannot be induced by the glucocorticoids to increase the MTV RNA content although these cells have other functions clearly under hormonal control. Although the cDNA probe is not well characterized and may not represent a copy of the entire viral genome, the results observed thus far suggest that MTV RNA produced by S49 cells is similar to that of GR cells. If this conclusion is correct, then the unresponsiveness of MTV in S49 cells implies that the chromosomal site of provirus insertion rather than the proviral sequences themselves may determine the hormonal sensitivity of the virus.

S49 cells are a θ-positive, TL-positive line of lymphoma cells which arose following mineral oil infection of a Balb/c mouse. They grow in suspension culture with a generation time of about 16 hours and clone (over a mouse embryo fibroblast feeder layer) with high efficiency in soft agar. They are growth-inhibited and subsequently killed by glucocorticoids and cyclic AMP. Steroid- and cyclic AMP-resistant variants have been selected and studied to determine the mechanisms by which hormones regulate cell growth. Cyclic AMP inhibits wild-type cells early in the G_1 period of the cell cycle. Cyclic AMP-resistant variants remain viable and display cell cycle parameters similar to their wild-type parents. These variants are entirely lacking the activities of the cyclic AMP binding protein and its associated protein kinase. Their existence demonstrates that cyclic AMP-dependent protein phosphorylation is not required for the orderly progression of events in the cell cycle. Even cells deprived of a functional cyclic AMP-stimulated kinase system, however, can be brought to arrest in G_1 by, for example, glucocorticoid-treatment or amino acid starvation. Since cyclic AMP cannot be responsible for this regulation, the role of cyclic GMP has been investigated in preliminary work. Thus far no antagonistic effect of added cyclic GMP has been observed when added to cyclic AMP- or steroid-arrested cells. These results, if confirmed, suggest that intracellular mediators other than the cyclic nucleotides may also regulate cell growth.

Kinetic studies have demonstrated that endogenously generated cyclic AMP progressively inhibits the cellular uptake and retention of labeled uridine, thymidine and 2-deoxyglucose. The uptake of these compounds which is

sensitive to endogenously generated cyclic AMP was not affected under the same conditions when a cAMP resistant mutant was studied. These results suggest that the kinase system is also responsible for controlling membrane transport. Nevertheless, detailed studies indicate that the inhibition of nucleoside transport is more rapid than that of the glucose analog (50% inhibition occurring before 4 hours with uridine and later than 12-15 hours with 2-deoxyglucose). These results imply the existence of at least several separate transport mechanisms, each of which is under cyclic nucleotide control. During the course of these studies it was observed that exogenous dibutyryl (db) cyclic AMP can inhibit uridine and thymide uptake and retention even in cAMP resistant mutants. That is, under circumstances where the endogenous cyclic nucleotide has no effect. Furthermore, under identical conditions db cyclic AMP has no effects on cellular growth. Therefore, diminished rates of nucleoside transport cannot be a determinant of cellular growth rates under these conditions. In addition, db cyclic AMP must possess pharmacologic properties not related to its activity as a cyclic AMP precursor.

A series of variants only partially resistant to the killing effects of cyclic AMP have been isolated. One of these contains maximal cyclic AMP binding and protein kinase activities equal to the wild type. However, they require about ten times as much added dibutyryl cyclic AMP to bring about growth arrest and cell killing as do normal S49 cells. Preliminary studies on the cyclic AMP-dependent kinase system indicate that kinase activation likewise requires a 10-fold increase in cyclic AMP concentrations to achieve maximal stimulation. Direct binding studies show that the affinity of the cyclic AMP binding protein for cyclic AMP is reduced a comparable amount with respect to the wild-type protein. Very preliminary experiments also suggest that the variant binding-kinase protein has a different thermolability than the wild-type protein when measured in crude extracts. These results strongly suggest that the variant protein has different physical properties than the wild-type molecules and very likely an altered amino acid sequence. If this is the case, and if the selection procedure does not uncover another wild-type gene for the kinase system, normally repressed in S49 cells, then it would be concluded that the variants are very likely mutational in origin. Similar conclusions have been reached on the basis of the examination of certain steroid-resistant S49 cell variants, as well.

Significance to Biomedical Research and the Program of the Institute:

There is considerable evidence that the entire genome of both DNA and RNA oncogenic viruses may be integrated into the chromosomes of host cells, whether or not such cells show evidence of their presence. A major aim of viral oncology is to understand the biological factors which regulate virus gene expression. Hormones are tissue-specific effectors which selectively control gene expression in a variety of cell types. Understanding hormonal regulation of viral gene expression may ultimately aid in understanding human cancer etiology and control.

Proposed Course: Work in the comming year will focus on how hormones control the expression of the murine mammary tumor virus genome. A line of cultured

mouse lymphoma (S49) cells synthesizes MTV RNA but provirus expression is not glucocorticoid sensitive in these cells, compared with GR cells. The reasons for this difference will be sought. GR-S49 cell hybrids will be constructed to attempt to determine whether MTV regulation or its absence is dominant. Experiments comparing the glucocorticoid receptors and their nuclear uptake will be carried out in both cell lines. If possible, cell-free transcription systems from GR and S49 cells will be prepared, and by mixing experiments, attempts will be made to define the differences between the two systems.

Date Contract Initiated: April 25, 1972

Current Contract Level: $70,000

UNIVERSITY OF CALIFORNIA (NO1 CP 33283)

Title: Studies on the Putative Role of RNA Tumor Viruses in Human Tumor Cells

Contractor's Project Director: Dr. Marcel Baluda

Project Officer (NCI): Dr. Leo Phillips

Objectives: 1) To extend the findings of Gross leukemia virus (GLV) and Simian sarcoma associated virus (SSAV) DNA specific sequences in human DNA; 2) To characterize these virus specific DNA sequences and determine whether they are transcribed in human tumors; 3) To characterize new isolates of promising candidate human oncornaviruses, and to determine whether they can be used as RNA or DNA probes for the detection of putative human oncornavirus specific DNA sequences; and 4) To investigate whether, in cancer patients, the oncornavirus DNA sequences are restricted to neoplastic cells or uniformly distributed in all tissues and whether transcription of virus specific DNA sequences is limited to the tumor cells.

Major Findings: Leukemic cells transformed by AMV contain new AMV-specific DNA sequences which were not present before infection.

In cultured chicken embryo fibroblasts infected with avian sarcoma or leukemia virus, viral DNA (vDNA) synthesis was detected intracellularly within 1 hour after infection, and vDNA integration into cellular DNA within 24 hours. There was a several-fold increase in the vDNA content of infected cells during the initial phase of virus infection. But, only a part of this newly synthesized vDNA appeared to become covalently linked with high molecular weight cellular DNA. In normal chicken cells, endogenous viral DNA appears to be associated with cell sequences reiterated 1200 times, and each integration unit appears to have a maximal size approximately equivalent to the 35S RNA subunit of the virion. In AMV infected cells, additional viral sequences are found which reassociate as if they integrated adjacent to unique cellular DNA.

A kinetic complexity of 3.3 X 10^6 daltons was obtained for the 35S AMV RNA. This value is very close to the estimated molecular weight of a 35S subunit. Therefore, all the 35S subunits are either identical or differ only slightly.

The AMV RNA sequences which hybridize rapidly in DNA excess are not restricted to any one region of the AMV 35S RNA. They appear to be randomly distributed over the entire RNA molecule and some are positioned within 200 nucleotides of the poly (A) tract at the 3'-end of the RNA.

In liquid hybridization experiments with an excess of human cellular DNA the 30S Gross Leukemia Virus RNA subunit (obtained by heat denaturing 60-70S RNA) hybridized to the same extent (2-3%) with DNA from leukemias, solid tumors, normal adult spleens, or four of seven apparently normal placentas. However, with DNA from two placentas, 10% of the large GLV-RNA subunit hybridized and with DNA from one placenta 46% of the 30S GLV-RNA hybridized. A pool of the four GLV-RNA subunits of intermediate size (9S-25S) hybridized significantly (7%) only to DNA from the same three human placentas which hybridized the most with the 30S subunit. The smallest GLV-RNA subunit (4-5S) hybridized equally (11%) to DNA from all neoplastic or normal human tissues. DNA from GLV producing mouse cells hybridized about 80% of each GLV-RNA subunit except the 4-6S subunit which hybridized only about 50%. Between 37 and 44% of each subunit hybridized to DNA from normal mouse cells.

DNA from normal and neoplastic human tissues contain sequences complementary to the RNA of a Woolly monkey oncornavirus (SSAV-1) produced in human rhabdomyosarcoma cells. An average of 43% of the viral 50-60S RNA hybridized with DNA from four different types of neoplastic tissues (bladder carcinoma, hystiocyticreticulosis, adenocarcinoma and acute myelogenous leukemia). DNA from the spleen of a patient who succumbed to generalized atherosclerosis, but in whom at autopsy a cortical renal adenoma was noticed, hybridized 58% of the viral RNA. An average of 23% of the SSAV-1 RNA hybridized to DNA from two normal human placentas while 50% of the SSAV-1 RNA hybridized to DNA from SSAV-1 producing human tumor cells.

SSAV-1 propagated in human rhabdomyosarcoma cells contains little 50-60S RNA but relatively large amounts of human ribosomal RNA, as well as 28-30S, 18-20S and 4-10S RNA. These smaller virion associated RNAs lack poly(A). The 50-60S viral RNA has a guanine plus cytosine content of 56 percent.

Significance to Biomedical Research and the Program of the Institute:

This study will tell whether human cancer cells contain putative oncornavirus specific DNA that is not present in normal cells, and the distribution of such DNA among the various tissues. It thus will provide important information as to whether oncornaviruses are associated with human cancer.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: June 8, 1972

Current Contract Level: $183,750

UNIVERSITY OF CALIFORNIA (NO1 CP 43212)

Title: Comparative Studies on the Structure and Replication of Murine and Avian RNA Tumor Viruses

Contractor's Project Director: Dr. Peter Duesberg

Project Officer (NCI): Dr. Robert Bassin

Objectives: To study the RNA structure and the replication of avian and murine RNA tumor viruses.

Major Findings: The RNAs of four different murine sarcoma-leukemia virus complexes (Kirsten, Friend, Harvey and Moloney) could be resolved into two species: One species had the same electrophoretic mobility as the RNA of helper leukemia virus, the other was smaller on the basis of its higher electrophoretic mobility than the leukemia RNA standard. Preliminary experiments using comparative electrophoresis and fingerprinting of electrophoretically purified RNA components indicate that the large RNA components of sarcoma-leukemia complexes are identical to the RNAs of pure helper leukemia virus. Preliminary experiments indicate that the electrophoretic mobilities of the small RNA components, present in the defective sarcoma-leukemia virus complexes, are different. Kirsten sarcoma virus contains the RNA with the lowest electrophoretic mobility and Friend-, Harvey- and Moloney sarcoma virus, in this order, contain RNAs of higher mobilities. Comparative fingerprint studies of the electrophoretically purified small RNA components, of two of the four transforming viruses (Kirsten and Moloney) indicate that they are little related to each other. However, preliminary studies suggest that the fingerprint patterns of the small, presumably transformation-specific, and the large, probably helper leukemia-specific, RNA components of the Harvey sarcoma-leukemia virus complex are related.

The template activity of the 60-70S RNA complex and of the 30-40S subunit RNA species of Rous sarcoma virus (RSV) was tested in a cell-free protein synthesizing system from mouse ascites Krebs II cells. Stimulation of protein synthesis over the endogenous background was about two-fold with 30-40S RSV RNA and about 1.3-fold with 60-70S RSV RNA as template. Analysis by SDS-gel electrophoresis showed that the predominant polypeptide synthesized in vitro in response to 30-40S RSV RNA had a molecular weight of 75-80,000 daltons. This polypeptide could be precipitated by antiserum against the group-specific (gs)-antigens of RSV, although its molecular weight is higher than that of virion-gs-antigen proteins. Analysis of tryptic digests of the in vitro-made protein indicated similarity to tryptic digests from authentic virion-gs-proteins.

Analyses of the RNAs of viral recombinants led to the conclusion that recombination among avian RNA tumor viruses involves crossing over.

By electron microscopy, it was found that 60-70S RNA of Prague Rous sarcoma virus had an approximate average weight of 6 x 10^6 daltons and contained predominantly two 30-40S subunits held together at many points in a network

structure. The size of the 30-40S RNA was determined to be around 3 x 10^6 daltons which is in good agreement with earlier determinations of the molecular weight of 30-40S RNA obtained by other methods.

Using the RNAs of avian tumor viruses as a model, the complexity of several wild-type and recombinant RNAs was determined to be around 3.5 x 106 daltons based on analyses of unique, RNase T_1-resistant oligonucleotides of viral RNA. It is concluded that the 60-70S RNA of avian tumor is polyploid, consisting of several identical 30-40S subunits.

Significance to Biomedical Research and the Program of the Institute:

Basic research on RNA tumor virus structure and replication will provide the basis for understanding viral carcinogenesis which may ultimately lead to the control of human cancer. Studies on the subunits of 60-70S RNA may pinpoint the localization of the virally transmitted oncogenic information.

Proposed Course: Work in the coming year will focus on (1) attempts to determine the gene order of tumor virus RNA by (a) sequencing viral RNA; (b) translation of viral RNA; and (c) analysis of DNA fragments obtained by digestion of proviral DNA with restriction enzymes; (2) study of the relationship of the small and the large RNA components found in murine sarcoma-leukemia virus complexes; and (3) study if host modification of viral components is detectable if the same virus is propagated in different hosts.

Date Contract Initiated: June 29, 1971

Current Contract Level: $111,822

UNIVERSITY OF CALIFORNIA (NO1 CP 43298)

Title: Regulatory Proteins in Oncogenic Viruses: Polyoma and SV40

Contractor's Project Director: Dr. Theodore Friedmann

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To study the temperature sensitive papovavirus mutants, polyoma ts3 and SV40 ts*101. These mutants have no virus-specific functions expressed at the non-permissive temperature. Infection of cells with the naked viral DNA bypasses the temperature-sensitive block, and leads to one normal cycle of replication. The favored hypothesis is that a mutant virion protein is responsible which does not get removed from the viral DNA. There is some evidence that these mutants are defective in maintenance of transformation. This is perhaps due to defective action of the same mutant protein. The objectives of the contract are to identify structural differences between the proteins of the wild type and mutant virions. Specifically, (1) isolate and purify each of the proteins from purified virions of wild-type polyoma and SV40 and the mutants ts3 and SV40 ts*101, (a) then examine each purified protein by peptide mapping and (b) do partial sequence analysis on any that are different; (2) attempt to find gentle conditions for partial

disruption of virions to search for DNA-protein complexes, (a) if found, examine by gel electrophoresis and (b) test for infectivity at permissive and restrictive temperatures; (3) characterize the DNA-protein interactions, (a) number of protein molecules per DNA, (b) whether the protein binds to one or both strands of the DNA, (c) fragment the viral genome with restriction enzymes and determine which fragment the protein binds to, (d) effect of protein binding on conformation of viral DNA, and (e) effect of protein binding on viral DNA transcription.

Major Findings: On characterization of the virion proteins by means of SDS-polyacrylamide gel electrophoresis, it appears that polyoma wild type and ts3 give identical gel patterns in this SDS system, despite an early indication that the 34,000 molecular weight protein of ts3 is electrophoretically slower than the protein of wild type virus. In similar experiments with SV40 wild type and ts*101, again, the early indication is that one protein in the low molecular weight histone-like region is present in the ts mutant but not in the wild type virus. The demonstration of this protein is difficult, since the resolution of the gel system in this molecular weight region is not reproducibly good. To improve this resolution problem, examination is being begun of the protein patterns on acrylamide gradient gels in the presence of SDS.

Attempts have been begun to develop other analytical gel systems; preliminary encouraging results have been obtained with 2 dimensional protein polyacrylamide gels using isoelectric focusing in the first dimension and the standard SDS system for the second dimension. The preliminary method has allowed the separation and identification of the non-histone proteins of wild type polyoma. Likewise, using marker proteins, the acid urea gel system of Chalkley has been adapted and improved to be used for the 1st dimension of a 2 dimensional protein pattern.

Whole purified virions and isolated purified major capsid proteins and the 34,000 and 21,000 molecular weight proteins from polyoma wild type virus and ts3 have been examined by 2 dimensional tryptic peptide patterns. The peptides of ts3 are indistinguishable from wild type virus.

Significance to Biomedical Research and the Program of the Institute:

This study should confirm or rule out the suggestion that the altered viral function which characterizes the temperature sensitive mutants, polyoma ts3 and SV40 ts*101 is reflected in a mutant virion protein. If it can be established by infectivity studies that a unique virion protein confers temperature sensitivity onto infection and transformation of cells with viral DNA, or if electrophoretic differences between wild type and temperature sensitive virions can be shown, then both the wild type and mutant proteins can be purified and characterized by standard methods of protein chemistry. Since the ts3 and ts*101 functions are important in the regulation of growth control in productively infected cells and in the expression of the transformed cells, identification and characterization of such a purified regulatory protein would be of great importance in understanding the mechanism of viral oncogenic transformation, and in providing clues for the involvement of

regulatory proteins in the development of neoplasia. A detailed understanding of the DNA protein interaction in these mutant oncogenic viruses could eventually lead to a development of antagonists or inhibitors of growth regulatory molecules involved in the development of neoplasia.

Proposed Course: Contract will terminate September 31, 1975

Date Contract Initiated: April 1, 1974

Current Contract Level: $49,000

UNIVERSITY OF CALIFORNIA (NO1 CP 53504)

Title: Studies on the Viral Etiology of a Malignant Lymphoma Outbreak in Rhesus Monkeys.

Contractor's Project Director: Dr. JaRue Manning

Project Officer (NCI): Dr. Wade Parks

Objectives: To study the epidemiology and suspected viral etiology of an outbreak of spontaneous malignant lymphoma in rhesus monkeys at the California Primate Research Center, at the University of California, Davis. Specifically: (1) To conduct a detailed epidemiological analysis of the lymphoma outbreak; (2) To examine sera from high risk animals for changes in serum proteins and compare with sera from animals with lymphoma and/or with appropriately matched control animals and to periodically monitor high risk animals and identify those with changes in serum β and γ globulins and total proteins which may be potential lymphoma cases; (3) To examine serum samples from lymphoma-bearing and high risk monkeys for antibodies to simian oncornaviruses and Epstein-Barr virus and virus-associated antigens; (4) to detect and characterize oncornavirus and herpesvirus-like particles and their associated antigens in spontaneous lymphoma tissues; (5) To propagate lymphoma-derived cells in tissue culture and screen for the presence of herpesvirus-like and oncornavirus-like particles utilizing immunological and biochemical methods; (6) To isolate and characterize type C viruses or herpesviruses detected in lymphoma tissues or cell cultures; (7) To transmit the disease to homologous and heterologous hosts.

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute:

The spontaneous outbreak of malignant lymphoma in macaques which will be studied on this contract is the first such outbreak recognized in nonhuman primates. There are a number of observations of the disease in the rhesus monkey which parallel lymphoma in man, particularly Burkitt's lymphoma. As in man, the etiology of this neoplasia in the rhesus monkey is unknown. Evidence concerning Burkitt's lymphoma in man, indicates that perhaps both a herpesvirus, and an oncornavirus are involved. Both may be involved in the

outbreak of lymphoma in nonhuman primates to be studied on this contract. Obtaining critical information on the etiology and immunobiology of this fascinating outbreak of malignant lymphoma in rhesus monkeys may appreciably enhance our understanding of lymphoma in man.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: September 23, 1974

Current Contract Level: $74,388

UNIVERSITY OF ILLINOIS (NO1 CP 43318)

Title: Studies on the Molecular Mechanism of Carcinogenesis by Oncogenic Viruses

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. Robert Bassin

Objectives: The work continues to focus on 1) human papovaviruses, especially BK virus, and 2) a cold-sensitive mutant of polyoma virus.

Major Findings: A mutant of polyoma was cold-sensitive for transformation of BHK cells. The permissive temperature was 38°C and the non-permissive, 32°C, with a differential between the two temperatures of approximately 5 to 7 fold. This differential remained consistent over a dose dependent range of transformation from 5 to 60 pfu/cell. The mutant appears to be temperature sensitive not only for the establishment of transformation, but also for maintenance. It appears that host cell DNA synthesis is induced by the cold mutant at both the permissive and non-permissive temperatures of the lytic cycle. For rat fibroblast cells, there was no cold sensitive transformation by this mutant.

In addition to being able to transform BHK hamster cells, BK virus can also transform the marmoset, diploid, fibroblast cell line, HF. The efficiency of BK virus transformation at any multiplicity is higher for BHK cells than for HF cells. The BK transformed HF cells grow in soft agar and reach a saturation density 4-6 times greater than untransformed HF cells. Further, although normal cells reach a stationary phase in culture and remain stable, the transformed clonal lines overgrew due to loss of contact inhibition with progressive cell detachment.

Evidence was found of cross-reacting cell surface antigens in cells transformed by papovaviruses of rodent (polyoma), or primate (SV40 or BK) origin.

Co-electrophoresing the polypeptides of BK virus with those of SV40 and polyoma virus showed that the major virion protein VP1 of BK virus was smaller than that of SV40 and even smaller than that of polyoma virus. VP3 of BK virus and of SV40 were identical in molecular weight. There was little similarity between the tryptic digests of VP1 of SV40 and BK virus and none at

all between those of VP3 of the two viruses. However, there was substantial similarity between VP4, VP5 from the two sources consistent with the claim that these may be histones, coded for by the cell genome.

BK virus infected HEK cell cultures routinely appear normal until 6-7 days P.I. at which time minimal CPE is demonstrable. No further cytopathogenic changes occur until the 9th-11th day when the cultures rapidly progress to maximal CPE. These observations indicated that either BK replication was much slower than that of other papovaviruses or that only a low percentage of the cells became initially infected and that several rounds of infection were necessary to produce sufficient virus to infect the balance of cells.

Twenty-one human cell types, either cell lines or primary culture were examined for the susceptibility to BK infection and production of infectious BK virus. The WI-26 established line and the F-998 (ATCC) lung fibroblasts proved to be the best for the growth of BK virus.

Significance to Biomedical Research and the Program of the Institute:

Studies on small icosahedral DNA viruses such as SV40 and polyoma have contributed an impressive body of information in recent years on the interactions between these viruses and cells. The genetic analysis of the genome of these viruses, by the use of mutants may provide a major advance in our knowledge of the mechanism of transformation at the molecular level.

These studies have recently become of great importance with the isolation of human papovaviruses, such as BK virus. BK virus is important as a potential candidate oncogenic virus for the human population, since: (1) It is capable of malignant transformation of rodent and primate cells. (2) It is of human origin, not closely related antigenically to polyoma or SV40. (3) It appears as a widespread infectious agent in the human population based upon hemagglutination inhibition serum studies.

Proposed Course: The main areas to be studied in the coming year are 1) a mutant of polyoma virus which is cold sensitive for the maintenance of transformation will be investigated in its interactions with different cells (hamster, rat, marmoset) to elucidate the role of cellular functions in the expression of mutation; 2) the BK virus biological system will continue to be developed and new strains will be investigated; and 3) the BK genome presence or absence in human tumors will be investigated.

Date Contract Initiated: December 9, 1971

Current Contract Level: $118,000

UNIVERSITY OF MINNESOTA (NO1 CP 33357)

Title: The Search for Tumor Virus Related Information in Human Immunodeficiency Patients with Cancer

Contractor's Project Director: Dr. John Kersey

Project Officer (NCI): Dr. Bernard Talbot

Objectives: To search for viruses in cancer patients with immunodeficiency diseases, to study B- and T- cell functions in cancer patients, and to study the role of the cell surface in malignant transformation.

Major Findings: Lymphoblastic leukemias and lymphomas have been studied from 30 children; in about 50% of instances malignant cells carry markers of thymus-derived lymphocytes. In some cases lymphoblasts form rosettes with sheep erythrocytes; in additional cases lymphoblasts carry the human T lymphocyte antigen detected using heterologous antithymocyte sera. In no cases have lymphoblasts from children with typical lymphoblastic leukemia or lymphoma been found which carry markers of B lymphocytes. Lymphomas in most children are of T cell origin. Adult lymphomas are in most instances of B cell origin. Childhood acute lymphoblastic leukemias are almost invariably of T cell or null cell origin. Two B cell malignancies were observed in childhood; both were "stem cell" or "American Burkitt's" lymphoma on clinical examination. Preliminary results indicate that children with T cell malignancies present with higher white counts, more frequently have mediastinal masses and have a worse prognosis than those with "null" cell malignancies. Implications concerning a possible RNA viral etiology of this group should be further pursued because of similarities with the virally induced T cell malignancies in the mouse.

Evaluations performed on a group of 12 adult patients with leukemia and lymphoma, at the time of diagnosis, following induction chemotherapy, after the completion of intensified therapy and then monthly for a period of 6-12 months showed the following results: The absolute numbers per mm^3 of T cells are usually either increased or decreased at the onset of the disease. When remission is obtained by induction chemotherapy, percentages of T lymphocytes return to normal values and they tend to stay at the normal level during several months of intensified and maintenance treatment. On the other hand, B lymphocytes are most often normal in absolute numbers at the time of diagnosis, but their percentages drop significantly following radio- and chemotherapy. This decrease is sustained during maintenance therapy.

Studies continue of patients with primary immunodeficiency diseases who develop cancer, currently directed at 1) determining the risk of malignancy in heterozygotes, 2) studying immunologic function in heterozygotes, and 3) developing family studies to look for histocompatibility antigen-linked immune response gene defects in homozygotes and carriers. In one family in which two siblings and a paternal aunt developed acute lymphoblastic leukemia, using HL-A testing, a chromosome was identified carried from the father to one leukemic child and two "normal" children. Further, the leukemic child and the two "normal" children all have evidence of a defective immunologic system as determined in the T cell rosette assay. It seems likely that histocompatibility antigen-linked immune response genes will be significant in cancer susceptibility as is clearly the case in the mouse.

Membrane lipids become increasingly fluid in the early minutes following mitogen binding to lymphocytes. These data, obtained using nitroxide labeled

fatty acids and electron spin resonance, are consistant with the notion that within five minutes following binding of mitogen, increased fluidity of membrane lipids may result in increased activity of membrane-bound enzymes, as for example, Na-K ATPase.

One patient with combined (T and B) system immunodeficiency developed a lymphoreticular malignancy following use of transfer factor. This lymphoid malignancy is now in cell culture, was sent to investigators within the Virus Cancer Program, and is available to others. Tumor material will probably soon be available from several other known patients with immunodeficiency syndromes who are currently bearing cancers for immunologic and virologic analysis.

Significance to Biomedical Research and the Program of the Institute:

The premise behind initiation of this contract was the idea that there should be a better chance of recovering a complete human tumor virus from a tumor in an immunologically incompetent patient than in an immunologically competent one. The contract provided information and materials from patients either with immunodeficiency diseases or iatrogenically immunosuppressed, which were investigated for the presence of tumor viruses.

Basic studies on B- and T- cell functions and on the role of the cell surface in malignant transformation may ultimately lead to ways to control the process of malignant metastases.

Proposed Course: Contract will terminate on July 12, 1975

Date Contract Initiated: May 13, 1971

Current Contract Level: $167,250

UNIVERSITY OF WISCONSIN (NO1 CP 53515)

Title: Studies on the Role of RNA Tumor Viruses and Related Genetic Information in Induction of Tumors by Chemicals

Contractor's Project Director: Dr. Robert C. Nowinski

Project Officer (NCI): Dr. Peter Fischinger

Objectives: 1) To investigate the role of MuLV and its gene products in spontaneous leukemia and in chemically mediated oncogenesis. 2) To study the natural humoral immunity of mice against murine leukemia virus.

Major Findings: The C3H/10T1/2 cell line, established from mouse embryo fibroblasts, is highly sensitive to post confluence inhibition of division and is susceptible to malignant transformation by chemical carcinogens in culture. Normal, chemically transformed, and spontaneously transformed clones of 10T1/2 cells were examined for the production of infectious MuLV and MuLV and MTV antigens. Criteria for the expression of oncornaviruses

included the production of: (1) MuLV and MTV gs antigens, (2) MuLV G_L and G_T cell surface antigens, (3) DNA polymerase-containing particles, and (4) infectious virions. Normal C3H/10T1/2 cells were free of oncornaviruses by all four of these criteria. Transformation of these cells to malignancy by chemical carcinogens did not result in an increased production of MuLV or MTV products; hence, these cells demonstrated the same virus-free phenotype as the parental cell line. Attempts to rescue transforming viral information from transformed C3H cell lines by superinfection with leukemia viruses were unsuccessful. Repressed endogenous MuLV (but not MTV) could be induced from all cell lines by treatment with IUdR. Induction of MuLV in normal cells occurred in three phases: I, an initial transient production of low-titered virus; II, a three to four week interval where virus production fell to a lower level; and III a resurgence of high-titered virus production. Transformed cells were more sensitive to treatment with IUdR than normal C3H/10T1/2 cells. These cells underwent a more rapid induction of MuLV, contained a larger fraction of cells than expressed MuLV functions, continued the production of MuLV throughout the second phase of induction, and had an earlier onset of the third phase of high-titered MuLV production. Oncornaviruses induced from IUdR-treated cells showed diversity in their host range and in their ability to produce XC plaques; none of these viruses, however, were capable of transforming normal C3H/10T1/2 fibroblasts in culture.

Mice from a wide variety of inbred strains formed natural antibody against endogenous MuLV. Mice of some strains (I, SJL/J, NZB, and NZW) produced high titers of antibody while mice of other strains showed either intermediate titers (DBA/2, C3H/An, HSFS, 129, GR, and AKR) or relatively low titers (C58, C57L/J and PL/J) of viral antibody. In general, older mice contained higher titers of antibody; however, there were many exceptions to this rule. These antibodies occurred in the γM, γG, and γA immunoglobulin classes, although the highest titer antibodies were of the γG_1 and γG_2 types. Mouse antibodies were directed against four of the six major viral proteins. Two of these proteins (gp70 and gp45) were glycosylated and were constituents of the viral envelope. One of the non-glycosylated antigens (p15) also was presumed to be located on the surface of the virion, although it was not considered to be as prominantly exposed as the two glycoproteins. The fourth viral antigen (p10) was known to be situated in the interior of the virus and was the arginine-rich ribonucleoprotein of the viral core. A demonstrable immune response to proteins p30 and p12 has not been observed. Notable variation was observed in the immune response of certain mice. For example, the γG_1 and γG_2 immunoglobulins of an NZB serum precipitated gp45 and p10, whereas the γM immunoglobulins from the same serum precipitated gp45, p15, and p10. This was in contrast to the serum of an NZW mouse, where antibodies (in all immunoglobulin classes) were directed against proteins gp70, gp45, and p15.

Significance to Biomedical Research and the Program of the Institute:

Carcinogenic chemicals can cause cancer in humans, mice and other animals. It is important to determine what role, if any, viral genetic information might play in this process.

Proposed Course: Contract will terminate on August 31, 1975

Date Contract Initiated: September 1, 1971

Current Contract Level: $287,597

6. OFFICE OF PROGRAM RESOURCES AND LOGISTICS

Dr. Jack Gruber, Chief, OPR&L, OADVO, DCCP, Chairman
Dr. Robert Holdenried, OPR&L, OADVO, DCCP, Scientist Director
Dr. David McB. Howell, OPR&L, OADVO, DCCP, Executive Secretary
Dr. Lea I. Sekely, OPR&L, OADVO, DCCP, Staff Scientist

CALIFORNIA, UNIVERSITY OF (NO1-CP-3-3237 and NO1-CP-4-0201)

Title: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses

Contractor's Project Directors: Dr. Stewart Madin
Dr. Adeline Hackett
Dr. Walter Nelson-Rees

Project Officers (NCI): Dr. James Duff
Dr. Jack Gruber

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biomedical Research Laboratory (NBRL), in Oakland. The program of the CCL is funded by a contract (NO1-CP-3-3237) between the University of California and the NCI. In addition, maintenance and operating expenses generated by the CCL are repaid to NBRL by an interagency transfer of funds (NO1-CP-4-0201) between NCI and NBRL. This project includes the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karyotyping of cell cultures, and performing biophysical, virological, and cytogenetic applied research.

Major Findings: During the past year, the contractor distributed 485 stock cultures to 95 recipients; initiated cultures from tumor and/or normal tissues from 25 donors; continued cultivation of cells, antibiotic free, and with low mycoplasma contamination (3% of 202 cultures); and revised, amplified, and prepared a new catalogue of available human and animal cell lines (Sept. 1, 1974), including cells at The American Type Culture Collection and low passage cells from exotic animals.

The contractor is continuing emphasis on growing differentiated cells from normal tissues and tumor cells from carcinoma and sarcoma specimens, with particular attention to morphological description of abnormal cells and correlation with tumorigenicity and product chemistry of preferred cells. To date, 131 specimens have been processed; 45 carcinomas, 35 sarcomas, and 22 miscellaneous and benign specimens were successfully grown, 24 specimens were discarded due to slow growth, and 5 specimens discarded due to contamination. The contractor will continue to study possible tumorigenicity of skin cultures from osteogenic sarcoma patients.

The contractor is carrying out characterization of human mammary epithelial cells by a) PAP stain, b) growth habits, c) nuclear/cytoplasmic ratio, and

d) virus production and EM fine structure. In addition, the contractor is continuing study of surface topography of human cells and possible viral agents associated with them by immuno-scanning electron microscopy. The contractor has defined specific cell-membrane associated antigens, topography of osteogenic sarcoma cells, lymphocytes associated with sarcomas, and the attachment properties of transformed cells.

Virus-host cell interaction studies have revealed replication of MMTV in normal mouse cells. SV40-induced transformation is expressed to varying degrees as tumorigenicity in vivo or viral transformation of BALB/3T3 cells in vitro. The contractor has revealed a considerable incidence of intra- and inter-species cellular contamination in cell cultures being utilized in the Program. Of 69 cultures tested, 19 (28%) were non-concordant with purported origin. Chromosome banding patterns of other established cell lines are being accumulated and studied.

Significance to Biomedical Research and the Program of the Institute: The contractor has developed an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators and VCP contract laboratories. The contract continues to develop techniques for the identification and study of tumor cells oriented toward a study of the fundamental biology of tumor cells and the interaction between tumor cells and viruses of oncogenic importance.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; continue a reference laboratory for karyology of cells in culture; continue applied research in the biology of tumor viruses.

Date Contract Initiated: October 1, 1962

Current Annual Level: $462,919 and $83,700

CHICAGO PARK DISTRICT, LINCOLN PARK ZOO (NO1-CP-3-3271)

Title: Marmoset Breeding Colony

Contractor's Project Director: Dr. Lester E. Fisher

Project Officers (NCI): Dr. Robert Holdenried
Dr. Lea I. Sekely

Objectives: To provide marmosets in a quantity and quality sufficient for the needs of the research on tumor viruses conducted under Contract NO1-CP-3-3219 with Rush-Presbyterian-St. Luke's Medical Center.

Major Findings: This contract provides a marmoset breeding colony at Lincoln Park Zoo in order to furnish live, healthy baby animals for collaborative research studies in viral oncology carried out at the Rush-Presbyterian-St. Luke's Hospital in Chicago.

There are presently 133 adult animals in the colony. During this reporting period there were 47 live births and 10 infants which were dead on delivery. Deaths in the breeding colony totaled 10, the primary causes including respiratory and enteric disorders.

There are no changes anticipated in the contractor's operations for the next two months. However, owing to renovation of the zoo and other problems relating to internal operating space, the contractor anticipates termination of his activities in September, 1975.

Significance to Biomedical Research and the Program of the Institute: This contract is part of a program utilizing lower primates for testing selected laboratory specimens for oncogenic activity. The marmoset, a small, inexpensive primate, has been shown to be susceptible to several cancer viruses; newborn and young animals are in demand by VCP investigators.

Proposed Course: At the contractor's request, this effort will be terminated in September, 1975, due to a necessary and unavoidable reorganization of the contractor's facilities.

Date Contract Initiated: June 28, 1965

Current Annual Level: $22,675

CHILD RESEARCH CENTER OF MICHIGAN (NO1-CP-3-3333)

Title: Inter- and Intraspecies Identification of Cancer Cells In Vitro

Contractor's Project Director: C. S. Stulberg, Ph.D.

Project Officer (NCI): Dr. David McB. Howell

Objectives: This contract provides Virus Cancer Program investigators with a service for rapid establishment or confirmation of species identity of cell culture systems.

Major Findings: In the past twelve months, 148 cultures were examined by 291 separate procedures that utilized genetic markers of three general kinds, i.e., isozymes, species antigens, and chromosomes. Other markers were employed as required. Results were reported to submitting investigators usually within ten days after receipt.

Approximately 35.1% of the cell lines were found to be either of a species or intraspecies origin different from that designated by the submitting investigator. This rate of misidentification or contamination is substantially the same as that found and reported last year. Most of the cultures submitted (77.5%) were of human tumor origin. Other species of cell lines examined included: marmoset, Rhesus monkey, Cercopithecus monkey, mouse, rat, cat, dog, sheep, Syrian and Chinese hamster, rabbit, bat, horse, and mink.

Particular emphasis was placed on developing cytogenetics procedures, particularly trypsin-Giemsa banding of human chromosomes. Work also continued on antigens of cell lines derived from T cell lymphomas.

Significance to Biomedical Research and the Program of the Institute: In the search for oncogenic viruses, many cell cultures from the same or different species are used concurrently, which offer frequent opportunities for cross contamination. In multiple-species tumor transplantations, the species derivation of induced tumors sometimes comes into question. Generally, the significance of virus presence in tissue cells, the ability to grow virus, or the validity of virus isolator systems are all dependent upon assurance of the identity of the cell cultures used.

Proposed Course: The contractor will continue to provide service to The Virus Cancer Program and its contractors by rapidly determining interspecies and intraspecies identity of cell culture systems. Additionally, the contractor will extend and develop marker systems in accordance with needs for identification.

Date Contract Initiated: June 26, 1973

Current Annual Level: $75,000

UNIVERSITY OF COLORADO MEDICAL CENTER (NO1-CP-3-3400)

Title: Collection of Neoplastic Tumor Specimens

Contractor's Project Director: Dr. William E. Hathaway

Project Officers (NCI): Dr. Lea I. Sekely
Dr. David McB. Howell

Objectives: To obtain tissues and serum specimens from patients with various types of malignancies for collaborative studies carried out within the VCP.

Major Findings: During the past year, 823 tumor and control specimens and relevant patient information were obtained and sent to investigators within the VCP as directed by the Project Officer. Specimens were available from a wide variety of patients with diagnoses including leukemia, lymphoma, rhabdomyosarcoma, osteosarcoma, various carcinomas, neuroblastoma, and a variety of brain tumors. In addition, sera from close relatives of patients were collected whenever possible and a variety of normal tissues were made available to interested investigators.

Fresh and frozen tumor tissue (mostly malignant but some benign) from surgical procedures and autopsies were sent to the NCI-Litton Bionetics Resources Processing Laboratory and to the NCI repository at Flow Laboratories, Inc., for storage and distribution to collaborating investigators.

Additionally, large amounts of normal and patient sera were procured and sent to the VCP serum bank at the Flow Laboratories repository.

Under Program direction, the contractor has placed new emphasis on procurement of large amounts of fresh, heparinized blood from new leukemia and Hodgkin's disease patients. Special requests from VCP investigators for cord blood, body cavity fluids containing tumor cells, lymphocytes, and brain tumors (fresh tissue) were filled.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract of major importance to tumor virus research, since it is a primary source of diverse cancer specimens for NCI and other VCP researchers. A continuing supply of such specimens is absolutely necessary to the pursuit of the viral etiology of human cancer.

Proposed Course: Continue to collect serum, blood, and tumor specimens as in the past. Materials will be provided to the VCP at the direction of the Project Officer for use in investigations by collaborating investigators.

Date Contract Initiated: June 18, 1969

Current Annual Level: $126,000

DOW CHEMICAL COMPANY (NO1-CP-3-3243)

Title: Review and Development of Biohazards Control and Containment Facilities

Contractor's Project Director: Mr. Cyril B. Henke

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives: 1. In collaboration with the NCI Office of Biohazards and Environmental Control, the contractor performs biological safety and environmental control surveys of VCP contractor operations to evaluate laboratory practices, safety equipment, and facilities, and to assess the effect of contractor operations on laboratory safety and environmental quality.

2. The contractor assists the NCI Office of Biohazards and Environmental Control in the preparation and dissemination of safety equipment specifications, facility design criteria, operations guidelines, and safety procedures. The contractor also prepares engineering design drawings and specifications for containment systems required to meet specific program needs.

3. The contractor operates a safety equipment certification program to assure safe operation of VCP safety and containment equipment.

4. The contractor assists the Office of Biohazards and Environmental Control in the review of plans and specifications for VCP renovation and new facility construction projects.

Major Findings: During the past year the contractor has continued to provide biohazards and environmental control consultation services to the NCI Virus Cancer Program which included the evaluation of biohazards associated with equipment and procedures, the performance of safety surveys, and the dissemination of relevant safety information. During the last twelve months, the contractor's effort has been directed toward:

a) Evaluation of pre-production laminar flow safety cabinets in a cooperative procurement endeavor with the Division of Research Services, NIH, Central Operations Branch, NIH, and the National Cancer Institute.

b) Further development of safety cabinet test methods and manufacturing specifications for NIH and NCI.

c) Conducting biological safety and environmental control surveys of NCI contractor laboratories.

d) Continuation of hazard assessments relative to operation of centrifuges and other laboratory equipment.

e) Continuance of support to the NCI safety and environmental control program through generation and dissemination of pertinent information.

f) Further development of safety training programs consisting of audio-visual combination units and short courses.

g) Development, procurement and testing of prototype containment equipment.

h) Providing technical consultation in addition to engineering design and review services on an as-needed basis.

i) Field certification and modification of laminar flow biological safety cabinets.

Significance to Biomedical Research and the Program of the Institute: This contract contributes biological safety and environmental control expertise to the VCP. This expertise is used to improve the quality and safety of the cancer research laboratory environment. The contractor functions as an integral part of the NCI Office of Biohazards and Environmental Control.

Proposed Course: The contractor will continue to provide technical assistance to the VCP contractors on problems of environmental control, personnel safety and product protection. The contractor will participate in approximately ten safety and environmental control surveys. The contractor will review all plans and specifications for renovation and new facility construction projects upon request. All recommendations will be based on compliance with accepted engineering design, biological safety

and environmental control practices. The contractor will continue to prepare the slide-cassette tape packages and provide distribution to VCP contractors.

Date Contract Initiated: June 25, 1965

Current Annual Level: $125,000

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-4-3351)

Title: Biological Resources Management Information System

Contractor's Project Director: Mr. Edwin Lisiecki

Project Officers (NCI): Ms. Wilma Varrato
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: The major objective of this contract is the implementation of a computerized central inventory system for the various resources of The Virus Cancer Program.

Major Findings: The progress achieved on this support contract may be described by summarizing the activities for:

- Central Inventory System (CIS) and related subsystems

- Satellite Systems Support

The CIS and related subsystems area included the addition of inventory data from the University of Illinois, Hektoen Institute, Memorial Hospital for Cancer and Allied Diseases, and the Simpson Memorial Institute. In some cases these additions required simple and complex modifications to existing computer programs, while in others new utility programs had to be written to perform the necessary data conversion and encoding functions on the new data for integration into the CIS data base.

In addition, subsystems have been designed for the purpose of generating intelligible management reports. These include a Virus Resource Management system and Activity Management reporting system. These subsystems are currently undergoing final development and implementation.

Finally, the CIS has been maintained in such a manner as to continue to be operated efficiently and economically by adjusting programs and data files in order to process the ever growing inventory data base.

The satellite systems support area includes the maintenance of the Naval Biomedical Research Laboratory (NBRL) inventory system and the Huntingdon Research Center (HRC) interactive inquiry/response system. In both systems normal maintenance has been provided as required; however, in HRC, the

design and implementation of an update function with supporting documentation have been completed.

Significance to Biomedical Research and the Program of the Institute: The necessary expansion of the inventory of viruses, sera, tissue cultures, human specimens, and other materials used in cancer research makes it vital that close control be exercised over these resources. Computerization of the inventory will eventually make it possible for the NCI Office of Program Resources and Logistics to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction, thereby permitting rapid response to the needs of the Program while avoiding resource excesses or shortages.

Proposed Course: The contractor will continue to extend and refine the central inventory system. Additional management reports will be generated for VCP resources control. Additional work on implemented subsystems will include: regular collection of new data; maintenance of automated subsystems; addition of interactive updates of test and bleeding data in Huntingdon Research Center's production of antisera. Work on new subsystems will be scheduled by OPR&L as needed.

Date Contract Initiated: May 3, 1971

Current Annual Level: $145,000

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-3-3355)

Title: Development of Propagation Procedures, Purification, and Characterization of Viruses

Contractor's Project Director: Mr. John Lemp

Project Officers (NCI): Dr. George Todaro
Dr. Jack Gruber

Objectives: To develop propagation procedures to produce high virus yields from cell cultures, and to purify and otherwise characterize the produced virus.

Major Findings: During the past year the contractor has grown, processed and characterized 4,256 liters of virus-containing fluid harvested from 30 cell lines as directed by the Contract Project Officer. The purified virus concentrates and packed tissue cells were distributed to the Project Officer and to individual investigators involved in a variety of research projects. The flexibility of the program is indicated by the fact that all of the above 30 cell lines, which shed C-type virus particles, were new to the contractor's laboratory when they were received from the Project Officer. In many cases, the yield of virus per tissue cell was increased during propagation procedures in the contractor's laboratory, through experience and in-house investigation. As part of an effort to continually

increase the quantity and improve the quality of the oncogenic viruses, the contractor successfully developed a 9-liter glass spinner flask with an aseptic withdrawal cock for the culture of cell lines in suspension. This year, as a result of in-house experience, seven of the contract virus lines were adapted to spinner culture, thereby increasing the virus yield and expanding the capability of the facility for producing more virus-containing fluids without affecting roller-monolayer capacity.

In addition to electron microscopy, the contractor utilized in-house capability for measuring total protein, RNA dependent DNA polymerase activity, acrylamide gel electrophoresis for identifying viral protein and RNA, antigen and antisera complement fixation titers, SC infectivity assay, and immuno-gel diffusion identity tests on the virus concentrates. These above mentioned biochemical and immunological methods are often used to select propagation procedures to improve yields under this contract and to provide comparable high-quality virus from many new and experimental cell lines.

Significance to Biomedical Research and the Program of the Institute: The search for evidence of the viral etiology of human cancer must include studies on viruses present in cell cultures established from animal tumors as well as on those candidate human cancer viruses growing in either animal or human cell cultures. Large volumes of these well-characterized and concentrated viruses are essential for the preparation of specific antisera and for the biochemical, immunological, and epidemiological investigations necessary in cancer virus research.

Proposed Course: This contract will continue to provide new and improved tissue culture propagation procedures and optimize methods for providing the highest yield with consistantly high biological qualities. In addition, the contractor will continue to characterize the virus-yielding cultures by thin-section electron microscopy when they are received from the researcher, and during propagation.

Date Contract Initiated: May 28, 1971

Current Annual Level: $361,000

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-2-3249)

Title: Large-Scale Production of Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To provide a service related to the isolation, large-scale production, concentration, and assay of oncogenic viruses of animals and potentially oncogenic viruses of humans. Production and quality control

involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: During the past year the contractor processed 7586 equivalent liters of virus-containing fluid harvested from several tissue culture systems and, as directed by the Project Officer, distributed the purified virus concentrates, cells, and special vaccine preparations to the Office of Program Resources and Logistics repository and to individual investigators involved in a variety of research projects. The flexibility of the program was emphasized in several ways: the high volume production and purification of AKR leukemia virus was terminated in favor of increased production of Gross leukemia virus; the Woolly Monkey sarcoma associated virus (KW-23) was propagated in a rat cell line (NRK); the Moloney leukemia virus was produced in the dense NIH-3T3 cell line obtained from Drs. W. Parks and E. Scolnick; and the Baboon ILN virus was produced from the new cell line obtained from Dr. Goldberg (Dr. Parks). Four viruses were produced from cell lines received from Dr. S. Aaronson and concentrated products were supplied to Dr. Aaronson and to Dr. R. Wilsnack (for antisera): NZB mouse virus/human A673 cells; MuLV-NIH mouse virus/human A673 cells; BALB mouse virus-2/Fisher rat cells and BALB mouse virus-2/human A673 cells.

In cooperation with Dr. R. Huebner, the contractor has developed a procedure which consistently yields vaccine preparations of AKR and Gross leukemia viruses with high CF titers. This procedure requires a freshly-harvested virus fluid, immediate centrifugation in a sucrose density gradient in citrate buffer, and seven-day, 1/4,000 formalin inactivation of the virus at 4^{o}C. This method, which has provided vaccine preparations with CF titers of over 1:1,000, should be applicable to other viral vaccines. The vaccine procedure has been recently modified.

Significance to Biomedical Research and the Program of the Institute: In order to carry out important research on the biochemistry and biophysics of oncogenic animal viruses, it is imperative that large quantities of concentrated virus be available for analysis. This contract helps meet this need with oncogenic animal viruses that have been produced under rigidly controlled conditions, and also serves to find the best means of producing and concentrating large quantities of new candidate human cancer viruses as they are discovered.

Proposed Course: The contractor will continue to provide Gross leukemia virus, Moloney leukemia virus, and special vaccine preparations, and will provide other special preparations as requested by the NCI project officer. The contractor will continue to develop and optimize methods for producing the highest virus yield with consistantly high biological qualities with the flexibility to quickly accommodate shifts in Program requirements.

Date Contract Initiated: March 27, 1972

Current Annual Level: $780,221

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-4-3334)

Title: Virus Processing and Production Facility

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officers (NCI): Dr. George Vande Woude
Dr. Jack Gruber

Objectives: The purpose of this contract is to establish and maintain a facility capable of a variety of batch-scale production of oncogenic and/or suspected oncogenic viruses; and also for the processing of large volumes of virus-containing fluids supplied by other NCI designated laboratories or investigators.

Major Findings: During the past year the contractor established the capability of growing relatively large volumes of various Herpes viruses (HSV-1; Patton, Mal, Syncytial Mal, and Syncytial V; HSV-2:333 and Rabson) in VERO cells, as well as growing Epstein-Barr virus (EBV) in HR1K cells and H. saimiri in OMK cells. The contractor also scaled up the procedure for polyethylene glycol (PEG) precipitation/Renografin gradient centrifugal purification of the Herpes viruses, and processed, labelled (when requested) and characterized 2,210 equivalent liters of virus fluids by the PEG/Renografin technique.

The contractor also grew, processed, and characterized many different viruses, including baboon placenta virus; genome-translation defective FeSV/FeLV; defective, "C"-type murine virus from BALB/c lines; and Kirsten MSV. Eight hundred seven liters of virus-containing fluids were concentrated and purified by conventional, sucrose-gradient banding centrifugal techniques.

In addition, the contractor received virus-containing tissue culture fluids, including Kirsten leukemia virus, Moloney leukemia virus, feline leukemia and sarcoma viruses, and baboon inguinal lymph node virus, from VCP collaborating investigators. These agents were concentrated by sucrose gradient density centrifugation. Other viruses supplied by collaborating investigators, including EBV, H. saimiri, and Kirby virus, were purified by the PEG/Renografin technique.

Significance to Biomedical Research and the Program of the Institute: This project provides cooperating investigators with the opportunity to process large quantities of virus-containing tissue culture fluids, as well as smaller quantities of a variety of viral agents of special interest. There is a strong need for this effort within the Program since it specifically relates to the determination of the possible viral etiology of human cancer.

Proposed Course: The work described above will be continued at the same level of activity.

Date Contract Initiated: June 15, 1974

Current Annual Level: $325,000

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NO1-CP-3-3343)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Robert Holdenried
Dr. Lea I. Sekely

Objectives: To monitor the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to VCP collaborators for transplantation, tissue culture and virus isolation studies.

Major Findings: During the past years a group of 85 aging and/or irradiated rhesus monkeys and progeny were monitored by daily observations and triannual physical and hematologic evaluation. These examinations specifically concentrated on the detection of developing neoplasms.

During the most recent survey, 14 animals were noted to be moderately emaciated, with the remainder of the animals in fair to good physical condition. Two animals have prominent proliferative lesions of the gums; three animals have ocular abnormalities; four animals were noted to have a palpable mass in the lower abdomen in the region of the uterus; and skin and/or subcutaneous nodules or lesions were noted in eighteen animals.

Two adults and one infant from this group died during the past year; one of the adults died of a widely disseminated fibrosarcoma, while the others died of causes not involving malignancy. Two animals from other sources--an adult female rhesus with an astrocytoma and a 42-year-old, male, pet capuchin monkey with a breast carcinoma--were donated to the contractor. Tissue samples from these animals were sent to Drs. Kawakami, Rabin, Wolfe, and Arnstein for investigations.

There have been no pregnancies in animals in the irradiated and/or aging rhesus group during the past year, owing primarily to the remodeling of the housing unit and consequent disruption of the breeding schedule.

Attempts to induce tumor formation by irradiating young rhesus monkeys and inoculating them with viable tumor cell suspensions from other monkeys have thus far been unsuccessful.

Significance to Biomedical Research and the Program of the Institute: The VCP conducts collaborative projects for the study of relationships between the etiologies of tumors of various primates. This project provided tumor tissues and other important specimens from aging non-human primates

which have been subjected to irradiation for research within the VCP. At the same time the contractor conducts a screening operation for the appearance of virus-like particles or viral antigens in the monkeys. Malignant changes in these primates may provide useful information which might be applied to humans, who are also subjected to various forms of radiation as well as to the natural aging process.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations. All tumors which develop will be evaluated by the contractor by light and electron microscopy. Specimens of these tumors will be made available to VCP investigators. In addition, a breeding program is underway to evaluate the incidence of leukemia or other tumors in infants with aging and irradiated parents.

Date Contract Initiated: May 1, 1971

Current Annual Level: $28,000

FLOW LABORATORIES, INC. (NO1-CP-3-3201)

Title: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens

Contractor's Project Director: Mr. Harry F. Adkins

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: To provide for the VCP a centrally located low temperature storage and distribution center for viral reagents and tissues.

Major Findings: During the current reporting period, the contractor made 555 shipments of viruses, viral reagents, sera, and tissues which comprised a total of 21,378 vials of material. He received 236 shipments of similar material which comprised 17,430 vials. All incoming shipments were carefully checked for damage in transit and were cataloged before being placed in the low temperature repository.

During the same period, the frozen tissue repository portion of the contract received 1,732 tissue and fluid specimens, most of which were from patients with neoplastic disease. One thousand two hundred and twenty two of these specimens were distributed to 20 investigators within The Virus Cancer Program. All tissue specimens were examined by Dr. Aloysius Kuo, a pathologist, classified as to tumor or tissue type, and either cataloged and stored or sent to VCP investigators.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible, adequately characterized resource materials. The laboratory, storage, and shipping facilities operated under this contract enable collaborating investigators to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: It is anticipated that the activities of this contract will continue to provide rapid and flexible support to changing needs of the VCP and its collaborating investigators.

Date Contract Initiated: June 22, 1965

Current Annual Level: $169,219

FLOW LABORATORIES, INC. (NO1-CP-3-3391)

Title: Animal Holding Facility to Support Intramural Research on RNA Viruses and Breeding for Detection of Tumor Virus Information.

Contractor's Project Director: Ms. J. Torgersen

Project Officer (NCI): Dr. John W. Pearson

Objectives: The objective of this contract is to provide a small animal breeding and holding facility to support intramural research activities. These activities require large numbers of mice, rats, and guinea pigs as well as smaller numbers of hamsters and rabbits for genetic studies, and animals for inoculation with various agents to be monitored for experimentation and observation during the aging process.

Major Findings: During the past year, this contract continued to support intramural research activities conducted at NCI in the Viral Biology Branch. The nature of the intramural activities required: (1) the long term holding of large numbers of mice for the development of spontaneous neoplasia during the aging process; (2) long term holding to study the effects of drug therapy, vaccines, non-specific immune stimulators and interferon used alone or in combination against viral-associated transplantable lines in aged mice, rats, and guinea pigs (200 days); and (3) the holding and breeding of selected mice to characterize hormonally influenced viral gene marker expression. During the past year, the contractor has received for either short or long-term holding a total of 7600 animals to support the above-mentioned activities (1) and (2). In addition, 11,000 weanlings have been obtained from selected strains of mice and shipped to NCI for genetic studies. A collaborative study continues with Dr. Joseph Coggin, the University of Tennessee, and the project officer involving the occurrence of spontaneous neoplasia in female AKR and C_3H mice following ovarian ligation. A certain number of these mice are being shipped periodically to Dr. Coggin for *in vitro* analysis. The remaining animals are being held at the contractor's site for monitoring of spontaneous

disease. Studies are continuing with Dr. Kalman Perk, a visiting scientist from Israel, to observe for the development of leukemia in guinea pigs following the inoculation of various preparations of cell-free virus materials. Finally, a repository has been established for frozen stocks of several mouse, rat, and guinea pig leukemia and tumor lines.

Significance to Biomedical Research and the Program of the Institute: This contract provides a support service which helps provide experimental data useful for determining the association and influence of viruses on the development of the neoplastic process. Investigations of this type utilizing chemo-immunotherapeutic and genetic approaches in both rat and mouse leukemia and tumor model systems, may result in information which will have considerable application in studies on similar diseases in man.

Proposed Course: Supportive activities related to the application of chemoimmunostimulation studies against the spontaneous AKR leukemia as well as the described rat leukemia systems will continue. Immunization studies involving the various rat leukemia cell lines will continue. Future work in this project will involve cross matings, backcross matings, and $F_1 \times F_1$ matings to characterize hormonally influenced viral gene marker expression.

Date Contract Initiated: June 15, 1971

Current Annual Level: $145,000

FLOW LABORATORIES, INC. (NO1-CP-5-3528)

Title: Virus and Reagent Production and Purification

Contractor's Project Director: Dr. Raymond Gilden

Project Officers (NCI): Dr. Robert Huebner
Dr. David McB. Howell

Objectives: To provide viruses and viral reagents to NCI intramural personnel and contractors engaged in a broad approach aimed at defining the role of viruses or genetically related elements in human cancer and intervention with their activity. Central to this function is the production of large amounts of type C viruses derived from different species and purification of these viruses by centrifugation techniques. In addition to the production of viruses and viral products, about 20% of the operation is also involved in producing viral vaccines for anti-cancer vaccine studies in a number of species.

Major Findings: This contract carries out the following tasks: a) growth and purification of large volumes of Type C viruses as specified by the NCI project officer; b) characterization of products by protein determination, particle counts, and quantitative immunoassays; c) distribution of virus internally and to collaborating VCP investigators as designated by

the project officer with the cognizance of the Office of Program Resources and Logistics; and d) inoculation of purified virus and viral subunits into suitable animals for production of antisera.

During the past year, the contractor has processed 13,439.2 liters of tissue culture fluid, with a final yield of 11,449.2 ml. of concentrated virus. These materials were derived from multiple cell lines and include viruses of mouse, rat, hamster, cat, baboon, Gibbon ape, Woolly monkey, and bovine origin. Among the agents produced are: AKR-V-1 virus; AT-124 virus; MMTV; BKD virus; Gross leukemia virus; Kaplan radiation leukemia virus; Graffi hamster type C virus; S-16 virus; Rauscher leukemia virus; and feline leukemia virus. The concentrates were used by personnel of contract NO1-CP-4-3387 for reagent production (e.g., neutralizing and component antisera) and supplied to investigators under contract NO1-CP-4-3388 for use in immunochemical and molecular hybridization studies. In addition, viral preparations were supplied to the Project Officer for vaccine studies and routine serological testing; also supplied were a variety of antisera. Viral concentrates and antisera were also supplied to Drs. F. Dixon, F. Jensen, Scripps Clinic; D. Bolognesi, Duke University; J. Hartley, NCI; M. Gardner, and J. Brown, USC; T. Aoki, NCI; P. Arnstein, NCI; Dr. A. Freeman, Akron Children's Hospital; Drs. Price and Parker, MAI; and several other investigators.

In addition to virus production activities, the contractor is continuing production of the following antisera: neutralizing antisera to Kaplan radiation leukemia virus; specific antisera to the gs antigen of the Graffi hamster type-C virus; specific antisera to the FL-74C strain of feline type-C virus; and antisera to the disrupted virus preparation of the endogenous baboon virus grown in dog cells (BKD).

Significance to Biomedical Research and the Program of the Institute: The materials provided by this contract are being applied to detect the presence of viral nucleic acids within human and animal tumor cells, to investigate viral and cellular contributions to neoplastic transformation at the molecular level, and to characterize the biological activity, antigenic composition, and relationship to known viruses of new agents isolated from human and animal tumor and other tissues.

Proposed Course: This contract will continue to provide NCI investigators with sufficient quantities of a variety of viral materials and antisera to permit continued intensive studies of the possible viral etiology of human cancer.

Date Contract Initiated: February 1, 1971

Current Annual Level: $820,000

FLOW LABORATORIES, INC. (NO1-CP-5-3529)

Title: Support Services to Maintain Studies on Herpesviruses

Contractor's Project Director: Dr. Jeffery G. Derge

Project Officer (NCI): Dr. Berge Hampar

Objectives: The objective of this contract is to provide support services for herpesvirus studies carried out by NCI personnel or under NCI direction at the contract facility.

Major Findings: During the past twelve months this contractor provided the following support services: a) preparation and formulation of tissue culture media; b) maintenance of human and non-human cell lines; c) large-scale production of cells; d) initiation of new cell lines; e) maintenance of animal facilities; f) maintenance of stocks of human and animal herpesviruses; g) routine serologic and biochemical assays for viruses; h) routine nucleic acid hybridizations; i) immunization of animals; j) routine immunofluorescent assays; k) electron microscopy; l) histology and pathology; and m) glassware services.

Significance to Biomedical Research and the Program of the Institute: The research program of the Solid Tumor Virus Section, Viral Carcinogenesis Branch, is concerned with determining whether herpesviruses play a primary or ancillary role in the induction of human tumors. The support services provided by the contract are not available to the NCI staff from other sources and are required for the continued operation of the program.

Proposed Course: It is expected that the contractor will continue to satisfactorily furnish all of the support services required for this project.

Date Contract Initiated: February 1, 1971

Current Annual Level: $377,000

GEORGETOWN UNIVERSITY (NO1-CP-3-3404)

Title: Supply of Blood and Tissue Specimens from Patients with Malignancies

Contractor's Project Director: Dr. Richard A. Binder

Project Officer (NCI): Dr. David McB. Howell

Objectives: To collect fresh blood and tissue specimens from patients suffering from various neoplasias.

Major Findings: Continued working arrangements with the Departments of Pathology and Medicine have been made for obtaining fresh operative tissue,

post-mortem tissue and serum from patients with neoplastic disease. Serial specimens, particularly of the latter, have been obtained during this period. Over the period involved in this report, a total of 305 specimens have been forwarded either to the NCI repository at Flow Laboratories, Inc. or the NCI Resources Processing Laboratory at Litton-Bionetics, Inc. This represents a compilation of 121 serial sera, 17 units of whole blood, 7 malignant pleural effusions, 25 malignant bone marrows, and 130 solid tumor malignancies.

Significance to Biomedical Research and the Program of the Institute: It is vitally important that a continuing supply of specimens from patients suffering from neoplasias be available to researchers seeking the viral etiology of cancer. This contract is particularly advantageous because the contractor is located within a few miles of NCI. This proximity allows the formulation and alternation as necessary of particularly detailed protocols, and also allows for the availability of very fresh specimens to VCP researchers in the Washington area.

Proposed Course: It is anticipated that increasing numbers of fresh tissues will be procured and provided to The Virus Cancer Program.

Date Contract Initiated: June 28, 1972

Current Annual Level: $25,000

HAZLETON LABORATORIES, INC. (NO1-CP-3-3212)

Title: Support Services to Maintain Studies on the Role of Viruses in Experimental Oncogenesis and Human Cancer

Contractor's Project Director: Dr. David Valerio

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: The objective of this contract is to provide services in support of research into the role of viruses in experimental oncogenesis and human cancer.

Major Findings: During the past year, this project provided necessary and essential services to support in-house research of the Molecular Biology Section, Viral Carcinogenesis Branch, concerning the viral etiology of human cancer. The functions included preparation of tissue culture medium according to rigid standards for sterility and cell viability as well as provision of freezer space for medium storage. The project produced specialized viruses and cells in large quantities for NCI staff and established and propagated human tumor cell lines and other specialized lines in tissue culture as reagents for the Viral Oncology Program.

The project also provided standardized assays according to established protocols for the detection and quantitation of type C RNA viruses in

samples supplied on a routine basis by NCI staff. These tests included radioimmunoassays for type C RNA viral polypeptides, the reverse transcriptase assay, RNA-DNA and DNA-DNA hybridization, and biologic assays including the focus forming assay for sarcoma viruses and the X-C plague assay for leukemia viruses. Electron microscopy was also available as a service on a limited basis.

Other services provided by the project in support of NCI in-house research included glassware facilities and care and holding of animals. The project provided facilities which strictly adhered to biohazard regulations of NCI. These included separate laboratories for work with different viruses, including those classified as moderate risk agents.

Significance to Biomedical Research and the Program of the Institute: This project provides essential functions for highly productive in-house research whose goals are to establish a viral etiology of human cancer and to develop methods by which to prevent this disease. The project also provides the Viral Oncology Program important resources including human tumor cell lines established in tissue culture, specialized viruses, and testing services for detection and identification of new virus isolates.

Proposed Course: The contractor has proven expertise in providing these services and has a very high probability of continuing these efforts at a superior level of performance.

Date Contract Initiated: September 1, 1972

Current Annual Level: $600,000

HEALTH RESEARCH, INC. (RPMI) (NO1-CP-4-3392)

Title: Procurement of Leukocytes, Whole Blood, and Tissue Specimens from Patients with Malignancies

Contractor's Project Director: Dr. Joseph Sokal

Project Officers (NCI): Dr. Lea I. Sekely
Dr. David McB. Howell

Objectives: To collect tissues and blood samples from adults suffering from neoplastic hematologic disorders, particularly Hodgkin's disease and leukemia, for use by researchers within the VCP.

Major Findings: Health Research, Inc. is located at the Roswell Park Memorial Institute in Buffalo, N. Y., one of the major centers for treatment of cancer in this country. The contractor shipped a total of 98 tissue specimens to the VCP tissue repository at Flow Laboratories during the past year. The aggregate weight of these specimens was 55,000 grams. The most common diagnoses represented were carcinoma of the lung, carcinoma of the breast, gastrointestinal carcinoma, gynecologic malignancies, and leukemia.

In addition, a total of 48 specimens of whole blood, 12 leukocyte concentrates, and 6 specimens of spleen were shipped to the NCI Resources Processing Laboratory at Litton Bionetics. Amounts of material shipped ranged from 10 ml. of whole blood to 15 x 10^{12} leukocytes, obtained by the NCI-IBM blood separator from a patient with chronic lymphocytic leukemia. The tumors represented included carcinoma of the lung, carcinoma of the kidney, carcinoma of the breast, reticulum cell sarcoma, lymphosarcoma, osteogenic sarcoma, fibrosarcoma, leiomyosarcoma, and a squamous cell carcinoma of the mouth.

Significance to Biomedical Research and the Program of the Institute: A major goal of the VCP is to identify, isolate, propagate, and characterize candidate human cancer viruses. Of paramount importance in the efforts to reach this goal is the continued availability of clinical specimens and histories from patients suffering from cancer. This contractor, which sees a large number of these patients annually, is in an excellent position to help meet increasing program needs for large quantities of human neoplastic specimens.

Proposed Course: It is anticipated that the rate of specimen acquisitions achieved during the past year will be increased.

Date Contract Initiated: June 22, 1972

Current Annual Level: $40,000

HEKTOEN INSTITUTE FOR MEDICAL RESEARCH (NO1-CP-4-3344)

Title: Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases

Contractor's Project Director: Dr. Paul Szanto

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The objective of this contract is to provide sterile frozen and viable normal or tumor specimens to collaborating laboratories within The Virus Cancer Program for biological, chemical, and virological investigations.

Major Findings: Since the initiation of this contract last year, a total of 105 sterile tissue specimens were shipped to the NCI Resources Processing Laboratory at Litton Bionetics. Diagnoses represented carcinoma of the breast, ovary, stomach, kidney, esophagus, malignant lymphoma and Hodgkin's disease, liposarcoma, and a number of benign neoplasms and normal fetal tissues. A total of 764 tumor and organ specimens were also shipped to the NCI frozen tissue repository at Flow Laboratories, Inc. The tumors represented included carcinoma of the bladder, carcinoma of the cervix, lymphomas, leiomyosarcoma, angioma, and carcinoma of the pancreas. Normal adult and fetal tissues, placentas, and benign tumors were also shipped.

Significance to Biomedical Research and the Program of the Institute: A continuous supply of appropriate human tissues from patients with cancer is vital to cooperative cancer research investigations. In order to carry out important studies on the biochemistry and immunology of suspected oncogenic human viruses, it is imperative that large quantities of human malignant tissues be available for analysis.

Proposed Course: This contractor will continue to supply tissue specimens from patients with neoplastic disease and from normal individuals.

Date Contract Initiated: June 17, 1974

Current Annual Level: $74,000

HOSPITAL FOR SICK CHILDREN (NO1-CP-2-3266)

Title: Human Normal and Leukemic Tissue Collection and Preservation

Contractor's Project Director: Dr. Peter D. McClure

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To obtain serum, whole blood, and tumor specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls.

Major Findings: The purpose of this contract is to supply serum and tissue samples from pediatric patients with leukemia, lymphoma, and solid tumors to NCI investigators and contractors. Serum samples and tumor material obtained at surgery or autopsy were frozen at -70°C and mailed at intervals to the NCI frozen tissue repository at Flow Laboratories, Inc. for long-term storage and retrieval. Buffy coats from acute leukemia were frozen at -70°C in DMSO and distributed through the NCI Resources Processing Laboratory at Litton Bionetics.

In the past twelve months the contractor continued to collect serum samples from new patients with leukemia and from patients on long-term follow-up for use by Virus Cancer Program collaborating investigators. Twenty-five tissue samples and seventy-one frozen and three fresh buffy coats from leukemia were shipped to the Litton Bionetics Resources Processing Laboratory for distribution, while sera from forty-one new leukemias, thirty-five follow-up leukemias, forty new tumor cases, twenty-one follow-up tumor cases, eighteen normal controls, and twenty-one hematologic controls, as well as thirty-one frozen tissue samples, were shipped to the NCI Flow Laboratories Repository. With the recent interest in obtaining large quantities of leukemic cells, attempts were made to isolate leukemic white cells greater than 1 gram, and deliver fresh specimens to NCI investigators within a 24 hour period.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor supplies many vital serum specimens to the VCP not readily available elsewhere. The contractor also provides an important service to Program by providing numerous samples of fresh and frozen buffy coats and tissue specimens. In addition, the contractor consistently refers patients to NCI for study whose cases are felt to be particularly relevant to the research needs of Program.

Proposed Course: The contractor will continue to collect solid tumor tissue, leukemic white cells and any other tissue specifically requested by NCI investigators.

Date Contract Initiated: February 3, 1965

Current Annual Level: $12,000

HUNTINGDON RESEARCH CENTER (NO1-CP-3-3223)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officers (NCI): Dr. Robert Holdenried
Dr. Wallace Rowe
Dr. David McB. Howell

Objectives: The objectives of this contract are to develop, produce, and characterize special diagnostic reagents for use in the VCP, primarily antisera and antisera conjugates to viruses, viral polypeptides, gs antigens, globulins of various animal species, and T-antigens of polyoma and SV40 in tumored hamsters.

Major Findings: The contractor during this reporting period produced and characterized antisera against a very wide array of antigens encountered in cancer research, and added a number of antisera to his inventory. Among the new antisera added during this period are goat anti-reverse transcriptase from Rauscher leukemia virus; goat anti-BALB 2 virus; goat anti-NZB virus; goat anti-baboon type C virus (BKCT); goat anti-RAV-0; goat anti-RSV, Prague strain; goat anti-BALB 2 virus, autologous cell origin; goat anti-reverse transcriptase from SSV-1; goat anti-p12 from Rauscher leukemia virus; Fischer rat anti-AT-124 virus; Fischer rat anti-BALB 2 virus; Fischer rat anti-BALB 1 virus; and goat anti-p27 from Mason-Pfizer monkey virus.

During this period the contractor has distributed approximately 15,500 ml. of antisera to over 145 collaborating investigators in the VCP. It is anticipated that this level of activity will continue.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed and produced by the contract are vital tools in cancer research. The project functions in close collaboration with VCP research projects and is of very significant usefulness to the needs of the program.

Proposed Course: To continue development, characterization, and production of antisera and serological test systems.

Date Contract Initiated: June 2, 1963

Current Annual Level: $345,912

UNIVERSITY OF ILLINOIS MEDICAL CENTER (NO1-CP-4-3345)

Title: Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases

Contractor's Project Director: Dr. Tapas K. Das Gupta

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The purpose of this project is to gather specimens of malignant human tumors and sera, pleural and ascites fluids, and whole blood from cancer patients as well as from normal individuals.

Major Findings: Since the activation of this contract in June, 1974, 384 tumors, 276 whole bloods, 66 sera, and 9 pleural and ascites fluids were shipped to the NCI Resources Processing Laboratory at Litton-Bionetics for disbursement to investigators.

Significance to Biomedical Research and the Program of the Institute: This project provides cooperating investigators with specimens from cancer patients in order to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: To continue to gather tissues and sera from human subjects with neoplastic tumors to be used by collaborating investigators within The Virus Cancer Program in the search for tumor-specific antigens and human oncogenic viruses.

Date Contract Initiated: June 17, 1974

Current Annual Level: $55,000

JEWISH HOSPITAL AND MEDICAL CENTER (NO1-CP-4-3251)

Title: Supply of Human Specimen Material from Patients with Chromosomal Abnormalities and Malignancies

Contractor's Project Director: Harvey Dosik, M.D.

Project Officers (NCI): Dr. Bernard Talbot
Dr. Lea I. Sekely

Objectives: To supply NCI research investigators with skin and tumor specimens, sera, and cell cultures from subjects with chromosomal abnormalities and related conditions to permit investigations designed to explore the possible relationship between such conditions and the etiology of human cancer.

Major Findings: During the past year the contractor continued to collect skin biopsies for chromosome studies and serum samples from controls and patients. Sterile and unsterile tumor specimens have been collected from biopsy and autopsy material. Skin specimens have been cultured at the Jewish Hospital and also sent to NCI for culture and tumor viral studies. In addition, one hundred fourteen serum samples have been frozen to provide a serum bank of normal and patient groups.

Cytogenetic studies have been performed on 15 long term human tumor lines. Giemsa Banding techniques and fluorescence were used to identify abnormal chromosomes and two lung lines were extensively studied. One hundred twenty-three skin biopsies, sixty-one frozen and seventy-two sterile tumor specimens were collected and cultured, distributed or stored.

Significance to Biomedical Research and the Program of the Institute: These studies enable the VCP to determine, on a broader scale, the relationship between chromosome anomalies (particularly those which involve an excess of genetic material), susceptibility to cellular transformation by oncogenic agents, and an increased incidence of malignancy.

Proposed Course: Continue to supply and study viral transformability of normal and neoplastic tissues from individuals with chromosome abnormalities.

Date Contract Initiated: March 1, 1973

Current Annual Level: $100,000

JOHNS HOPKINS UNIVERSITY (NO1-CP-3-3245)

Title: Pediatric Tumor Resource

Contractor's Project Director: Dr. Herbert Kaiser

Project Officers (NCI): Dr. Paul Peebles
Dr. Jack Gruber

Objectives: To provide fresh tumor specimens from pediatric patients to collaborating laboratories within the VCP for biochemical and virological investigations.

Major Findings: The material obtained during this twelve-month period included body fluids containing free tumor cells, solid tumor biopsy specimens, and both involved and uninvolved organs obtained at necropsy. In general most body fluids contained 10^7 to 10^9 tumor cells and most solid specimens were in excess of one gram of tissue.

All specimens were sent directly to the NCI Resources Processing Laboratory at Litton Bionetics, Inc. for disbursement to investigators of the Virus Cancer Program.

Significance to Biomedical Research and the Program of the Institute: Detection, treatment, and prevention of human cancer require an accurate determination of its etiology. Viruses have been implicated as possible causative agents in human cancer. Unfortunately, a lack of sufficient tumor material from patients in the pediatric age group limits investigations for oncogenic viruses that may have been vertically transmitted from mother to child. Materials from this contract will help make possible these imperative human studies.

Proposed Course: It is anticipated that this contractor will continue to supply unique pediatric specimens to the VCP.

Date Contract Initiated: March 1, 1973

Current Annual Level: $32,000

LIFE SCIENCES, INC. (NO1-CP-3-3210)

Title: Production of Germfree and Reagent Grade Specific Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar
Dr. David McB. Howell

Objectives: To produce both germfree and specific-pathogen-free (SPF) animals for cancer research. SPF animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts.

Major Findings: During the past year, three outbred expansion colonies of isolator-derived BALB/c mice were maintained in separate barrier-sustained cubicles. Each colony contained approximately 600 female breeders, with two colonies being used for supply and one for development. The BALB/c

colonies supplied 130 timed-pregnant, 839 adult, and 6,509 weanling mice to user laboratories. Each outbred colony was begun with small inbred stock maintained in a Reyniers isolator or a primary barrier cubicle. Antibody testing of BALB/c mouse sera indicated the line is free of 11 common murine viruses. One colony of 500 female, Caesarean-derived, outbred NIH Swiss mice supplied 131 timed-pregnant and 200 weanling animals to user laboratories, in addition to producing about 800 young breeders for development of the nude mouse colony. The outbred NIH Swiss nucleus was increased to 750 female breeders to meet increased demand for expansion of nude mouse colonies. Recent testing of NIH Swiss mouse sera from retired breeders showed no evidence of 11 murine agents in the closed colony. Three small, outbred expansion colonies of nude mice, derived from foundation stock reared in isolators, supplied 150 timed-pregnant, 187 adult and 305 weanling animals to The Virus Cancer Program. These colonies were expanded to increase the number of nude mice from 60 to 100 weekly. Developmental problems rearing and maintaining nude mice seem to be resolved. Breeding procedures and temperature were found to be critical in maintaining normal survival rates in the nude mouse mutant. A total of 4,617 fertile quail eggs and 230 young quail were supplied to user laboratories from a highly selected Japanese quail flock of 300 breeders maintained in a primary barrier cubicle. The isolator-derived flock was developed through elaborate trap nesting methods and recent monitoring in this laboratory indicates the line is free of common avian pathogens, including avian leukosis virus and Marek's herpesvirus. An isolator-derived, pedigreed chicken Supply Flock of 225 female breeders supplied 3,230 fertile eggs and 3,053 chickens during the year. The flock was developed from 45 pedigreed chickens which consistently demonstrated high productivity over a period of several generations. The pedigreed flock was monitored at regular intervals during the laying cycle and following retirement at 11-12 months of age. Extensive monitoring indicates the flock contain no avian leukosis virus, Marek's herpesvirus or other common avian pathogens. Regular phenotyping of eggs from pedigreed stock indicates presence of C/O and C/B types. In subsequent generations, the phenotype may change from one type to mixed thus requiring continual monitoring in each new flock to clarify status of each pedigreed bird. One small colony of Sprague-Dawley rats was kept at low level maintenance in view of no demand for timed-pregnant animals.

Significance to Biomedical Research and the Program of the Institute: This contract serves as an essential supply of embryonated eggs and day-old chicks to contract NO1-CP-3-3205, which involves studies on Marek's disease as a model for herpesvirus-associated oncogenesis. It also provides other VCP investigators with genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with a minimal danger of interference from other contaminating, adventitious microorganisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: This service-type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of

being reoriented as rapidly as possible to meet changing needs of VCP activities as they occur.

Date Contract Initiated: February 8, 1968

Current Annual Level: $390,454

LIFE SCIENCES, INC. (NO1-CP-3-3291)

Title: Production of Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. John W. Pearson

Objectives: The objectives of this project are the large-scale production in vivo of BAI strain A avian myeloblastosis virus (AMV) and the preparation and distribution of significant quantities of AMV reverse transcriptase enzyme.

Major Findings: During the past year, the contractor produced 566 grams of virus, 7,433 ml. of myeloblast cells, and 140,000 units of reverse transcriptase enzyme (RDDP) from the virus. During this same period, the contractor provided RDDP to 41 investigators, plasma-derived virus to 62 investigators, and myeloblast cells to 9 investigators. Approximately 2/3 of the recipients were in the United States and 1/3 in Europe and Japan.

The contractor has successfully completed the adaptation of AMV to growth in W36 strain chickens after long-term propagation in chickens of strain 934. It is anticipated that production of virus, cells, and enzyme will continue at approximately the same level in the coming year.

Significance to Biomedical Research and the Program of the Institute: One of the major objectives of the VCP is to explore fully all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced in vivo under controlled conditions which permit the study of the immunology, virology, biochemistry, and therapy of the tumor virus complex. Moreover, BAI Strain A avian tumor virus is the only RNA C-type virus which is at present available in large enough quantities to permit exhaustive investigation into the biochemical makeup and behavior of both the virus and its components. As such, it represents an important model for the C-type viruses of higher animals and is an essential tool in the search for cancer viruses in man. Future studies will depend upon large quantities of concentrated virus, which the contractor is uniquely in a position to supply.

Proposed Course: It is anticipated that the contractor will continue to meet requests for avian myeloblastosis virus and will continue the preparation of significant quantities of AMV reverse transcriptase enzyme.

Date Contract Initiated: April 19, 1971

Current Annual Level: $419,000

LITTON BIONETICS, INC. (NO1-CP-4-3224)

Title: Carcinogenic Activity of Selected Virus Preparations in the Newborn Monkey

Contractor's Project Director: Dr. David P. Martin

Project Officers (NCI): Dr. Robert Holdenried
Dr. Jack Gruber

Objectives: The objective of this contract is the maintenance of monkey breeding colonies and laboratories necessary for inoculation, care, and monitoring of primates.

Major Findings: During this report period, efforts continued to provide both New World and Old World primates and biological materials from these animals for use by investigators and to increase the size of the New World primate breeding colonies. A total of 110 New World primates comprised of two species was received to begin quarantine and conditioning. Six of these died, eight were assigned to experimental protocols, 96 were added to the breeding program, and 19 New World primates remained in quarantine and conditioning at the close of the report period. The New World breeding colony now has approximately 265 animals representing five species. During the report period, the renovation of the New World primate breeding area was accomplished, resulting in a complex of six rooms for the exclusive housing of New World primates. Approximately 358 macaques comprising three species are housed in the Old World primate breeding colony. Small breeding colonies of gibbon apes (Hylobates lar) and galagos (Galago crassicaudatus) are also maintained. One hundred forty-three live births occurred in the Old World primate breeding colony, and all of these were admitted to the Primate Nursery. Thirty-one known conceptions resulted in 19 live births in the New World primate breeding colonies during this report period, and of these, none were admitted to the Primate Nursery. A total of 57 animals were transferred from the Primate Nursery to the Primate Oncogenesis Section during the report period. Fifteen animals were transferred from the Primate Oncogenesis Section to other government projects, and at the close of the report period, 590 primates remained in the Primate Oncogenesis Section. In support of these efforts, personnel in the Department of Pathology performed 119 complete necropsy procedures and examined 116 biopsy specimens. This involved the preparation of 1,291 microslides (36 with special stains) and three frozen sections. The surgery team performed 13 major procedures to support the program, and the Department of Microbiology continued its

support by performing specimen cultures, antibiotic sensitivity testing, and environmental monitoring. The Hematology Section performed 2,412 complete blood counts and stored over 2,400 serum samples. One hundred sixty-two coagulograms, 21 clinical chemistry procedures, and 37 bone marrow specimens were processed. Routine monitoring and cataloging of specimens continued in the Parasitology Section. A total of 25 live primates and 38 tissue or serum samples were transferred or sent to outside investigators. At the close of the report period, work continued on 23 active special study programs for investigators as directed by the Project Officer.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes.

Proposed Course: This contract will continue the following activities: The maintenance of breeding colonies of macaques and the expansion and maintenance of breeding colonies of six different species of New World primates; production of 200 to 260 infants of various species of both Old World and New World primates for use in experimental studies; continuing efforts in the establishment of a breeding colony of gibbon apes (Hylobates lar); and the long-term holding and study of experimental animals inoculated by collaborative investigators and the independent research group. All of the systems needed for the production, hand rearing, isolation, and proper care of the primate species represented are included within this program.

Date Contract Initiated: February 12, 1962

Current Annual Level: $1,400,000

LITTON BIONETICS, INC. (NO1-CP-4-3260)

Title: Acquisition of Fresh Human Specimens for Virus Cancer Program Investigators

Contractor's Project Director: Mrs. Shirley Norris

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: The primary objective of this project is to coordinate collection, processing, and distribution of fresh human tissues from subjects with malignancies for Virus Cancer Program investigators under direction from NCI. Specimen needs are determined through requests from VCP intramural and collaborating investigators directed to the NCI Office of Program Resources and Logistics, and the contractor, through frequent

telephone contacts and personal visits, communicates Program needs to NCI contractors concerned with human resources collection to insure tissue acquisitions responsive to the Program needs. Additionally, this facility also maintains a serum bank for the VCP, provides tissue and serum inventory information to the OPR&L, and provides a monthly computer account of tissue and serum distribution and inventory.

Major Findings: During the reporting period, more than 1,283 specimens were picked up by messenger from Baltimore and Washington hospitals and airports and brought to the resources laboratory where condition of the specimens was observed, and sterile aliquoting was performed when samples were larger than required by a single investigator. Specimens were distributed to 26 principal investigators within The Virus Cancer Program. Additional serum collections were received from more than 1,187 donors and aliquoted for The Virus Cancer Program serum bank at Bionetics. The bank now contains aliquots from more than 3,500 different collections. Fresh specimens were delivered by messenger to investigators in the Baltimore and Washington areas. Air freight shipments were made to investigators at more distant locations. Investigators who specified to NCI a continuing need for fresh human tissue of a type which could be obtained were supplied with tissues on a regular rotating basis.

New efforts were undertaken to provide researchers with important materials. Repeated trips to Montreal and Toronto have been made to handcarry important specimens which could not have been shipped through customs rapidly enough to be useable as fresh materials.

Significance to Biomedical Research and the Program of the Institute: It is not sufficient merely that sources of human sera and tumor materials for cancer research exist; it is also necessary that the capabilities of these sources be coordinated with the needs of investigators who require specific, and often fresh, specimens. This contractor serves as a direct interface between user and supplier, occasionally sending personnel long distances to pick up specimens of unusual interest and perishability and to deliver them directly to the researcher, thereby avoiding the usual delays in shipment that might lead to the loss of an important factor or agent in the tissue.

Proposed Course: This project will remain responsive to changing program needs for fresh human tissues and attempt to increase amounts of important specimens collected.

Date Contract Initiated: November 1, 1973

Current Annual Level: $152,345

LITTON BIONETICS, INC. (NO1-CP-4-3249)

Title: Application of Animal Virus Model Systems to Human Neoplasia

Contractor's Project Director: Dr. Alan Rein

Project Officer (NCI): Dr. Robert Bassin

Objectives: The purpose of this project is to provide facilities and staff to support laboratory research investigations by intramural NCI scientists.

Major Findings: During the past 12 months the contract has supported NCI research investigators by the propagation of approximately 70 cell lines, each in a well-defined, stable condition as required for particular experimental uses. It has also performed in vitro infectivity assays for murine sarcoma and leukemia viruses, at a level of approximately 1,000 assay plates in the 12-month period. It has also carried out over 20,000 assays for DNA polymerases in virus and cell preparations, and 800 complement fixation and 1,000 radioimmunoassay tests for virus-related antigens.

Other support has included the production, characterization, and storage of new cell lines; production and characterization of virus stocks; fractionation of cell extracts for studies on virus-associated enzymes; and production and characterization of immune sera and immunocompetent cells. This effort contributed to 19 publications during the past contract year.

Significance to Biomedical Research and the Program of the Institute: An understanding of viral defectiveness and the role "helper" viruses play is of value in determining the occurrence and mechanism of viral oncogenesis by type C viruses in man. Identification of viruses or viral products in human breast tumor cells is of value in assessing the role of viruses in human breast cancer and, ultimately, in developing techniques of both diagnostic and therapeutic significance. This contract provides the support facilities necessary for the carrying out of this research.

Proposed Course: The contractor will continue to provide the following services: maintain cell cultures in an optimal state for virus production; carry out quantitative virus assays; recover virus-related products from the cells themselves; monitor cell cultures for virus production by a variety of specific biological assay techniques as well as by specialized enzyme assays.

Date Contract Initiated: June 27, 1969

Current Annual Level: $470,000

MELOY LABORATORIES (NO1-CP-4-3263)

Title: Mouse Mammary Tumor Virus Production Facility

Contractor's Project Director: Dr. Ron Gillette

Project Officers (NCI): Dr. Wade Parks
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: To propagate, concentrate, and distribute murine mammary tumor virus (MTV) for collaborating VCP investigators; to perform immunological and biological assays for the detection and quantitation of MTV; and to develop improved methods for the propagation and detection of MTV and MTV antigens.

Major Findings: The primary purpose of this contract is the production of quality reagents for the study of the mouse mammary tumor virus system as a model for the further examination of the human breast cancer problem.

The current RIII colony presently consists of approximately 3,000 breeding females, of which 2,000 are in the dairy facility. During this report period, major effort has been placed on increasing the size of the RIII mouse colony to about 3,000 breeding females in the dairy colony, thereby increasing the supply of lactating females for milk collection. Historically, the supply of milk has been adversely affected by seasonal changes in breeding rate. This problem should be eliminated with an increased supply of lactating females.

A total of 20 liters of RIII milk was collected during the report period and two hundred and eleven virus purification runs were completed yielding a total of 354 cc of purified mammary tumor virus. A total of 780 mammary tumors have been recorded and distributed. In addition, the contractor has investigated methods for increasing the breeding efficiency of the animal production colony.

The following antisera have been prepared and distributed: rabbit anti-C_3H-MTV, goat anti-RIII-MTV, goat anti-MTV gp·52, and goat anti-gp·27. MTV reagents have been distributed to over 50 investigators in the U.S.A. and also to 12 foreign laboratories.

Studies of methods of inducing MTV production in tissue culture have progressed. Use of halogenated steroids for this purpose appears promising.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. The finding of a virus, resembling a Type B RNA oncogenic virus of mice, in the milk of a significant number of women from high-risk breast cancer families strongly suggests a possible viral etiology for this disease. A major effort of The Virus Cancer Program is directed toward determining the relationship of viruses to human breast cancer. The contract was established

for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in humans may be developed.

Proposed Course: Purified MTV, viral reagents, and mouse milk will continue to be supplied as needed by VCP investigators. The contractor will also attempt to establish possible recipient epithelial cultures from normal mouse mammary glands as substrates for in vitro mammary tumor virus infection.

Date Contract Initiated: January 13, 1974

Current Annual Level: $410,000

MELOY LABORATORIES (NO1-CP-4-3236)

Title: Support Services for Immunological and Biochemical Studies of Mammalian Viral Oncology

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. Wade Parks
Dr. Edward Scolnick

Objectives: The purpose of this contract is to provide support services and facilities for studies on the immunology and biochemistry of mammalian viral oncology.

Major Findings: During the past year this contract provided essential services and facilities to support in-house research of the Viral Carcinogenesis Branch and the Viral Leukemia and Lymphoma Branch, VO, DCCP, concerning the viral etiology of human cancer. Among the services furnished by the contractor were: preparation and formulation of tissue culture media; maintenance of human and non-human cell lines; large-scale production of cells; initiation of new cell lines; maintenance of animal facilities; maintenance of stocks of human and animal oncornaviruses; routine biochemical assays for viruses; routine nucleic acid hybridizations; immunization of animals; routine immunofluorescent assays; electron microscopy; glassware services; and preparation of viral structural proteins and enzymes and cellular enzymes used for the study of RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The ability to detect viral information in transformed cells is basic to establishment of etiological association, and to an ultimate approach to prevention or treatment of cancer. This contract provides services and facilities essential to studies whose purpose is to develop this ability.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: May 25, 1965

Current Annual Level: $899,000

MELOY LABORATORIES (NO1-CP-4-3207)

Title: Support Services for Studies on Spontaneous and Virus Induced Neoplastic Transformation

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. George J. Todaro

Objectives: The objective of this contract is to provide facilities and support services for intramural studies on spontaneous and virus-induced neoplastic transformation.

Major Findings: During the past year this contract provided necessary facilities and services to support in-house research of elements of the Viral Leukemia and Lymphoma Branch, VO, DCCP, into the viral etiology of human cancer. Among the services furnished by the contractor were: a) preparation and formulation of tissue culture media; b) maintenance of human and non-human cell lines; c) large-scale production of cells; d) initiation of new cell lines; e) maintenance of animal facilities; f) maintenance of stocks of oncogenic and potentially oncogenic human and animal viruses; g) routine serologic and biochemical assays for viruses; h) routine nucleic acid hybridizations; i) immunization of animals; j) routine immunofluorescent assays; k) electron microscopy; l) histology and pathology; and m) glassware services.

Significance to Biomedical Research and the Program of the Institute: Important findings regarding the mechanism of action of oncogenic viruses, their effects on cellular growth control mechanisms and their possible involvement in natural oncogenesis have been developed from a number of projects on this contract. A number of new type C viruses have been isolated and characterized and have provided much information on the nature of type C virus-cell interactions. Hopefully, the techniques employed in inducing and growing these viruses may be used to isolate a complete human type C virus which would immeasurably aid in studies of the possible viral involvement in human cancer. The services afforded by this contract are of essential importance in furthering this research.

Proposed Course: To continue to provide services and facilities in support of the indicated research.

Date Contract Initiated: May 25, 1965

Current Annual Level: $1,194,000

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-4-3335)

Title: Acquisition of Human Specimen Materials for Use in Cancer Research

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

Objectives: To gather sera and tissues from human subjects with neoplastic tumors to be used in cancer research studies by collaborating investigators within The Virus Cancer Program.

Major Findings: This contract represents a major acquisition and distribution effort for the various human neoplastic specimen materials so necessary to the multifaceted research programs currently in progress seeking to ascertain the possible relationship of viruses to the etiology of human cancer.

During the past year, over 600 tissue samples were sent directly to various participating VCP investigators. Among the major beneficiaries were Drs. Spiegelman, Huebner, Pagano, Schlom, and Hanafusa. Direct contact was made with all participating laboratories at frequent intervals during the year and every effort made to tailor collection to actual laboratory needs.

Packing and shipping procedures were revised following consultation with the NCI Project Officer so that delivery of fresh human tissues within 24 hours is now routine and where necessary same-day arrival at the recipient research laboratory can be assured.

Furthermore, during this reporting period, 100 fresh, viable tissues and nearly 350 serum samples from patients with a variety of malignancies and 500 samples from normal blood donors were shipped to the NCI Resources Processing Laboratories at Litton Bionetics, Inc. for storage and general distribution.

Significance to Biomedical Research and the Program of the Institute: In the last ten years, rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City provides cooperating investigators with sufficient numbers of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: It is anticipated that this contractor will maintain the present high level of material supplied to the Program.

Date Contract Initiated: March 1, 1971

Current Annual Level: $165,000

MICHIGAN CANCER FOUNDATION (NO1-CP-4-3391)

Title: Collection of Large Quantities of Human Milk

Contractor's Project Director: Dr. S. Albert

Project Officers (NCI): Dr. E. Plata
Dr. David McB. Howell

Objectives: To collect large quantities of milk from women whose families have a history of breast cancer as well as from women whose families have no such history for use in cancer research investigations.

Major Findings: Human milk collected under this contract is sent to the NCI repository at Flow Laboratories, Inc. for general dissemination to collaborating scientists investigating the possible viral etiology of human mammary carcinoma.

During the first 12 months of the contract, the contractor's interviewers increased the number of hospitals visited from 2 to 5. These 5 hospitals account for 38% of the annual births in the metropolitan Detroit area. Over three thousand prospective nursing mothers were interviewed of which approximately 53% were from non-cancer and 47% from cancer families. Over twelve hundred milk kits were received from nursing mothers of which over 600 were from non-cancer and approximately 575 were from cancer families. Approximately 114 liters of identified human milk were shipped to the Virus Cancer Program Repository at Flow Laboratories, Inc. Nursing mothers from non-cancer families contributed 52 liters and those from cancer families contributed 62 liters.

Significance to Biomedical Research and the Program of the Institute: Particles with certain characteristics similar to animal oncogenic RNA viruses related to mammary carcinoma have consistently been detected in human milk; human milk is therefore currently a very important research material for studies on the etiology of human breast and other cancers.

Proposed Course: 1) To obtain identified, fresh aliquots of human milk in the amount of at least 200 liters, and to code, label, and ship those specimens to NCI designated investigators and the NCI Flow Laboratories repository as directed by the Project Officer. 2) To record familial cancer history and personal medical history in computer files for correlative milk and epidemiological studies.

Date Contract Initiated: June 27, 1974

Current Annual Level: $100,000

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-4-3254)

Title: Support Services for Studies of Type C RNA Tumor Viruses

Contractor's Project Directors: Dr. Damodar Deshmukh
Dr. Nirmal Mishra

Project Officer (NCI): Dr. Padman Sarma

Objectives: To provide support services for Type-C RNA tumor virus studies carried out by NCI personnel or under NCI direction at the contract facility.

Major Findings: Among the support services provided by the contractor during the past year were: a) preparation and formulation of tissue culture media; b) maintenance of human and non-human cell lines; c) large-scale production of cells; d) initiation of new cell lines; e) maintenance of animal facilities; f) maintenance of stocks of oncogenic animal viruses; g) routine serologic and biochemical assays for viruses; h) routine nucleic acid hybridizations; i) immunization of animals; j) routine immunofluorescent assays; k) electron microscopy; l) histology and pathology; and m) glassware services.

During this reporting period the contractor has satisfactorily furnished all of the support services required by NCI personnel.

Significance to Biomedical Research and the Program of the Institute: It is becoming increasingly apparent that many vertebrates, including man, carry information in their genomes for complete RNA tumor virus expression, including oncogenic potential. The studies supported by the services of this contract provide further information on the natural occurrence and spread of oncogenic type C viruses in homologous and heterologous species, including man.

Proposed Course: Support services will be continued for demonstration, isolation, and characterization of endogenous type C viruses of rat, cat, turkey, and man. Support services will also be provided for studies of the natural history of turkey reticuloendotheliosis virus and the isolation and characterization of bovine type C virus.

Date Contract Initiated: October 23, 1973

Current Annual Level: $370,000

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-3-3288)

Title: Mouse Virus Typing and Diagnostic Reagents

Contractor's Project Director: Dr. John C. Parker

Project Officers: Dr. Robert Holdenried (NCI)
Dr. Wallace P. Rowe (NIAID)
Dr. David McB. Howell (NCI)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent and cat viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory populations; and to assist in the characterization of the gene-dependent expression of murine leukemia.

Major Findings: The contract operates a murine virus serodiagnostic and viral diagnostic laboratory. During the past year, the serodiagnostic laboratory received 4, 859 sera on which a total of 39,239 tests were performed: 2,195 mouse sera (17,828 tests), 244 rat sera (2,108 tests), 213 hamster sera (1,266 tests), 77 guinea pig sera (539 tests), 68 cat sera (1,224 tests), 435 coded sera or antigens (1,174 tests) and 1,627 MAP test sera (15,100 tests). The virus diagnostic laboratory received tumor fragments, cell cultures, viral reagents and tissues from diseased rodents to test for possible virus contamination. Out of 121 specimens examined, 31 were found to contain one or more virus contaminants. Twenty-two specimens were positive for lactic dehydrogenase virus, 16 for minute virus of mice, 5 for polyoma, one for reovirus 3, and one for mouse hepatitis. Reagents for approximately 44 murine viruses and serotypes are available for diagnostic tests. These reagents in either the infectious or inactivated form are available on request for shipment to investigators. The contract has added a mycoplasma diagnostic capability to its diagnostic laboratory which will serve primarily to monitor cell lines, test viral reagents, and test tissues from diseased animals. The immunofluorescence capability has been expanded and the contract now can test for virus or antibody to 7 murine viruses.

During the past year, 2 significant viral epizootics in laboratory animals occurred, reemphasizing the need for continual surveillance of virus infections in laboratory animals. An epizootic of ectromelia virus infection in mice occurred simultaneously in 2 unrelated research institutes, one in Maryland and one in Wisconsin. In both instances, rapid diagnosis of ectromelia as the etiologic agent, and quarantine of the entire buildings, together with destruction of the infected colonies, prevented a panepizootic which could have crippled the research programs in the institutes. The second epizootic resulted when LCM virus became established in a commercial colony of hamsters from which infected hamsters were shipped throughout the United States. More than 100 human cases of LCM disease were attributed to contact with the infected hamsters before the colony was located and the hamsters destroyed.

A major responsibility of the contract is to provide special services to investigators who request assistance. More than 25 such special projects were completed this year. Some of the developmental and collaborative research projects are: (1) the description of a new parainfluenza virus

isolated from hamsters, (2) studies of the effects of host genetics on the expression of murine leukemia virus, (3) the effect of mouse thymic virus infection on the immunological competence of the mouse, (4) description of a neurological disease in mice induced by a Type-C leukemia virus, and other epidemiological and vaccine studies.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent and cat colonies and laboratory animal-produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems. LCM virus, in addition to being infectious for humans, is difficult to detect.

Proposed Course: To continue the serodiagnostic services outlined above and to improve the sensitivity of the tests. To apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

Current Annual Level: $462,000

UNIVERSITY OF MINNESOTA (NO1-CP-4-3285)

Title: Development of Training Courses in Microbiological Safety

Contractor's Project Director: Dr. George Michaelson

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives: The objective of this contract is to prepare for, and present, five times during the contract period, a training course entitled, "Principals of Biohazards and Injury Control in the Biomedical Laboratory".

Major Findings: During the past twelve months, four new courses were presented, two in June, one during September 17-20, and one during December 10-12, at the University of Minnesota and NIH. Thirty-three laboratory workers engaged in oncogenic virus research attended the first course which was held at the University of Minnesota in June. This represented the maximum number of students that could be accommodated because of the logistical limitation caused by the laboratory portion of the course. The second course was presented, without the laboratory, to 116 campus safety officers during a special session preceding the 21st National Conference on Campus Safety. The course was offered as a workshop to campus safety officers so that they would be fully aware of the laboratory safety practices the Office of Biohazards and Environmental Control is recommending for research involving oncogenic viruses. This training approach was taken to strengthen the safety support provided to university cancer research programs through their cognizant safety offices.

Significance to Biomedical Research and the Program of the Institute: The training program provides a most effective mechanism for disseminating current biological safety information. Course participants benefit directly by learning biological safety and environmental control methods and techniques. This program benefits the institute by increasing the general safety awareness of the laboratory participant and his associates.

Proposed Course: The first short course will be presented June 3-4, 1975 in Minneapolis. The second course is being scheduled as a collaborative training effort with the Tissue Culture Association and will be presented June 25-27, 1975 at the W. Alton Jones Cell Science Center, Lake Placid, New York. The Salk Institute will host the third course September 23-25 in La Jolla, California. Baylor University has agreed to host the fourth course, October 29-31, in Houston. The fifth and final course will be presented again at NIH in Bethesda, December 9-11.

Date Contract Initiated: December 10, 1971

Current Annual Level: $85,000

MONTREAL CHILDREN'S HOSPITAL (NO1-CP-3-3377)

Title: Procurement of Normal and Leukemic Sera from Children

Contractor's Project Director: Dr. Ronald L. Denton

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To obtain sera from a variety of pediatric oncology patients, family members, and controls for virologic study.

Major Findings: During the past year, 428 cases of serum and other special specimens were procured which included 39 new acute leukemia, lymphoma, and other solid tumor patients and 11 fresh placental tissues from pregnant patients with malignancies or from mothers whose previous child had leukemia.

A periodic shipping of frozen specimens to the NCI repository at Flow Laboratories, Inc. as well as special specimens to the Litton Bionetics Resources Processing Laboratory have been made.

Significance to Biomedical Research and the Program of the Institute: This contract is a source of serum from leukemic children and from suitable, normal controls. The increasing need within the VCP for samples of this kind makes it essential that the supply be continued to satisfy research requirements.

Proposed Course: Continue to collect serum, placenta, and tumor specimens for distribution to collaborating investigators within the VCP.

Date Contract Initiated: September 24, 1965

Current Annual Level: $40,000

NAVAL BIOMEDICAL RESEARCH LABORATORY (YO1-CP-4-0200)

Title: Aerosol Properties of Potentially Oncogenic Viruses

Contractor's Project Director: Dr. N. Vedros

Project Officer (NCI): Dr. A. Hellman

Objectives: This project involves applied research task areas as well as providing a standard hood-testing service to certain other NCI contractors. The task areas are: laboratory hazards from aerosols; environmental effects on characteristics of viral aerosols; host-virus interactions; and disinfectants.

Major Findings: During the past year, the contractor has been evaluating the survival characteristics of MSV and FeLV as a function of relative humidity (RH). It was determined that of the two RH studied (25% and 80%), recovery of viable virus was best at 25%, although even at this more optimum RH, 80% of viral activity, as measured by focus formation, was lost. In the case of FeLV, a similar phenomenon was noted.

Disinfectant studies using RLV have been completed and are being prepared for publication. Information gained from these studies has been transmitted to all contractors in The Virus Cancer Program. It is anticipated that standard procedures for adequacy of disinfectants will be prepared and provided to all contractors, so that they then will be able to determine the optimum conditions and disinfectants to employ in their particular situation.

Animal transmission studies and determination of horizontal spread of FeLV are currently in progress. Data thus far would suggest that the horizontal transmission of FeLV to adult cats is negligible. Studies with newborn and juvenile cats are currently under study.

Disinfectant techniques, propagation, and *in vitro* assay for *Herpesvirus saimiri* (HVS) are under progress. Disinfectant studies suggest that a 10-fold higher concentration of hypochlorite will be required to inactivate HVS than the type-C RNA viruses.

Physical studies of laminar hood performance are being continued as are support functions for west coast Virus Cancer Program contractors.

Significance to Biomedical Research and the Program of the Institute: An understanding of the survival and clearance rate for virus aerosol will allow the development of a rational and hopefully less expensive means for control of cross infection in laboratories conducting research in viral oncology. Similarly, development of mathematical models will permit the more precise evaluation of research facilities, on the basis of risk.

Proposed Course: To continue studies of specific problems in control of laboratory biohazards generated in work with oncogenic viruses and infected tissue cultures. In addition, to study synergistic effects of selected chemicals and viruses on oncogenic susceptibility, and the effect of physiological stress (as related to changes in immune response) on susceptibility. As adjuncts to this work, the contractor will study survival of selected airborne viruses in varied environments and optimize methods of assay.

Date Contract Initiated: March 1, 1971

Current Annual Level: $155,000

PFIZER, INC. (NO1-CP-3-3234)

Title: Large-scale Tissue Culture Virus Production for Cancer Research

Contractor's Project Director: Dr. Sami Mayyasi

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To provide a service facility for the production of large volumes of selected oncogenic and suspected oncogenic viruses, cellular antigens, tissue culture cell lines, and specific antisera to various oncogenic viruses. Production of these materials is supported by appropriate laboratory groups whose activities include process improvement, product standardization, quality control testing, and applied developmental research.

Major Findings: The current annual rate of large-scale tissue culture production results in 27,000 liters of virus harvest fluids and 1300 gms. of cells being processed to fulfill the needs of VCP investigators. During the past year, these materials were distributed in over 500 shipments to 100 laboratories throughout the world. The major products included: Woolly monkey sarcoma virus (SSV-1); Mason-Pfizer monkey virus (M-PMV); baboon endogenous virus (BV); and gibbon ape lymphoma virus (GALV). Other materials produced in smaller quantities to meet specific requests were: Rauscher leukemia virus (RLV); RD-114 virus; Epstein-Barr virus (EBV); feline leukemia virus (FeLV); MCF-7 (Rich agent); and products of the AO and J-96 cell lines (obtained by NCI from USSR).

Several developments in tissue culture production and virus processing techniques were investigated and new methods have been adopted to improve

the efficiency of manufacture and testing. Many of the oncornavirus or EBV-infected cell lines have been adapted to grow in 20L fermentors. Several oncornaviruses have been shown to replicate readily in a human lymphoblastoid cell line (NC-37). The biological, biochemical, and immunological properties of virus produced in NC-37 were shown to be of quality similar to that obtained when the virus is propagated in the parent cell line.

Significant progress has been made in the concentration and purification of oncogenic viruses. Preclarification of virus fluids is accomplished on a routine basis with J-21-B centrifuge fitted with a JCFZ rotor and is shown to result in a cleaner product. Molecular filtration has been introduced on a limited scale to concentrate large volumes of harvest fluids containing oncornaviruses before further purification by isopycnic banding in sucrose gradients. Multiple experiments have been conducted to demonstrate that 50 to 100 liter lots can be so processed without detrimental effect on the desired activity of the virus. By omitting the banding in sucrose gradients, the biological activity of concentrated EBV can be retained.

To assure purity and to quantitate the various products, several new assays have been perfected and can be applied routinely or when specified. These new techniques include the following: 1) spectro-photometric analysis of electrophoresed virus proteins; 2) identification of coded virus samples by their electrophoretic and immunological properties; 3) quantitation of gs activity by radioimmunoassay; 4) optimization of endogenous polymerase activity (RT) by selecting the proper concentration of the preferred divalent cation for each agent; 5) assaying the infectivity of endogenous baboon virus by counting syncytia produced in human glioma cell monolayers (KC line with RSV genome).

The new containment laboratory for quarantine and testing of foreign cell cultures was approved by the USDA and is now operating. Several cell lines from abroad have now been exhaustively tested by USDA approved methods (tissue culture, embryonated eggs and mice) to exclude foot and mouth disease virus and other undesirable agents prior to release of the cell lines to laboratories of VCP investigators.

Significance to Biomedical Research and the Program of the Institute:
Since its inception, this contract has been an invaluable back-up to intramural and collaborating investigators involved in virus-cancer research. The staff and facilities have been consistently responsive to changing needs of the NCI Virus Cancer Program. They have provided support to a wide variety of collaborating investigators making possible studies on viruses in cancer that could not otherwise be conducted.

Recent research developments to determine the association of viruses with human neoplasia have involved activities concerned with the molecular biology, biochemistry and immunology of oncogenic viruses. These types of investigations may provide clues to the mechanism whereby viruses mediate the transformation process. Such knowledge could indicate methods by which neoplastic transformation can be averted or inhibited and provide appropriate

control measures applicable to the human cancer situation. Studies of this type have created a substantial demand for large quantities of concentrated and purified oncogenic and suspected oncogenic viruses. This contractor has both the capability to help meet such needs and the flexibility to quickly accommodate shifts in Program requirements.

Proposed Course: The production of viruses and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961

Current Annual Level: $1,700,000

ST. JOSEPH'S HOSPITAL (NO1-CP-3-3393)

Title: Study of Human Sarcomas and Their Possible Viral Etiology

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officers (NCI): Dr. Albert J. Dalton
Dr. David McB. Howell

Objectives: To acquire and supply investigators within The Virus Cancer Program with fresh human sarcomas or other tumors which may contain EM evidence of virus particles, and to attempt to establish and supply cell cultures derived from such tumors.

Major Findings: During the past 12 months, 134 malignant tumors, 22 benign tumors, and 24 control tissues were shipped to the NCI frozen tissue repository at the Flow Laboratories, Inc. A total of 252 sterile human tissues were shipped to the NCI Resources Processing Laboratory at Litton Bionetics; these consisted of 139 malignant tumors, 113 benign tumors or other control tissues, and 3 placentas from first trimester. Nine tissue culture flasks were shipped to the project officer, Dr. Dalton, of NCI. Twenty-one sera from patients with malignant and benign tumors of the breast were shipped to the NCI Resources Processing Laboratory at Litton Bionetics.

New cultures were started from 19 tumors including 5 melanomas and 1 Wilms' tumor.

Significance to Biomedical Research and the Program of the Institute: This is an important project concerned with the search for viruses in human tumors. Extensive and careful examination by electron microscopy of a large number of human tumors and cell cultures established from these tumors is essential in determining the viral etiology of cancer.

Proposed Course: Collection of specimens, shipping to assigned laboratories, and screening of cultures of virus by EM and by appropriate immunological techniques will continue.

Date Contract Initiated: June 24, 1969

Current Annual Level: $65,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-3-3340)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officers (NCI): Dr. Robert Holdenried
Dr. David Howell

Objectives: To breed, maintain, and supply young and mature chimpanzees to investigators for research in the higher non-human primate concerning the possible viral etiology of human cancer.

Major Findings: The chimpanzees remain in a breeding situation. At present, no females assigned to this contract are pregnant; however, one infant was obtained from the Multiple Sclerosis colony in payment for a baby previously given to them.

During the year the chimpanzee colony had two deaths, chimpanzee #12 on September 29, and chimpanzee #60 on November 6, 1974. Infant #4-X16 was born to female #5 on this program and transferred to the Multiple Sclerosis program. However, infant X11 was born to Multiple Sclerosis mother #4-155 on October 4, 1974 and was assigned to this program.

Chimp X11 was inoculated with baboon C-type virus isolate No. 2162-2 and is under observation and study. In addition, infant 4-X26 was delivered by cesarean section as a germfree animal on December 13, 1974, and inoculated with Herpesvirus saimiri. This animal is being closely monitored.

Two additional chimpanzees obtained from the NCI primate facility at Litton Bionetics, Inc. were assigned to a program being undertaken by Dr. Carl Olson of the Department of Veterinary Science, University of Wisconsin.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. Newborn chimpanzees are particularly useful in determining susceptibility to suspected human cancer viruses because their resistance to virus infection is very low. This is the only source of newborn chimpanzees for the VCP.

Proposed Course: The chimpanzee colony will be maintained as before and newborn animals will continue to be supplied to investigators within the VCP.

Date Contract Initiated: April 25, 1969

Current Annual Level: $48,000

STANFORD UNIVERSITY (NO1-CP-4-3244)

Title: Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Director: Dr. Leonard Hayflick

Project Officers (NCI): Dr. James T. Duff
Dr. Jack Gruber

Objectives: To serve as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera, cell cultures, and clinical materials submitted by VCP contractors. Upon request, identification of isolates is made as to species; and mycoplasma antigens are distributed to those investigators requiring these materials.

Major Findings: During the past year, the contractor received and tested 1942 samples from VCP contractors for mycoplasma. Two hundred thirty-four positive isolations were made. In addition, at the request of several VCP-supported laboratories, the contractor ascertained the species of mycoplasmas found as contaminants in thirteen submitted samples. These were as follows: five isolates from Dr. Walter Nelson-Rees' laboratory at the Cell Culture Laboratory of the University of California, identified as M. arginini; three isolates from Dr. Janet Butel's laboratory at Baylor University identified as M. arginini; five isolates from Dr. M. Collins' laboratory at Microbiological Associates, including: 1 isolate identified as M. hominis, 1 isolate identified as A. laidlawii, and 3 isolates identified as M. pulmonis.

Several investigators within the VCP have need for mycoplasma antigens either isolated from their materials or the materials of others. This contractor has made these antigens and antisera available to several laboratories where they have usually been used for retrospective studies in which unusual observations were later found to be attributable to these contaminants.

A new biochemical procedure for detecting microbial contamination of cell cultures was developed. Studies are in progress on a mycoplasma inhibitor found in cell cultures that upon dilution reveals the presence of mycoplasmas which are masked in undiluted cell culture fluids and therefore yield negative results.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all VCP contractors and viral oncology intramural staff. Many of the most important viral specimens, cell cultures, sera, etc., used in the viral oncology program are sent to this facility for mycoplasma testing.

Proposed Course: This contract will continue to serve as a central diagnostic laboratory for the detection of mycoplasma contamination in virus pools, antisera, cell cultures and clinical specimens important to the VCP. Upon request, serological identification of isolates will be made as to species.

Date Contract Initiated: June 19, 1969

Current Annual Level: $50,000

UNIVERSITY LABORATORIES, INC. (NO1-CP-3-3222)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Dr. Robert Holdenried
Dr. Jack Gruber

Objectives: To supply highly standardized oncogenic viruses and their antisera in volumes necessary to meet VCP research needs.

Major Findings: The contractor's production of Rous sarcoma virus (Prague strain) in tissue culture roller bottles has continued at a very high level. During the past year, approximately 3,200 liters of virus-containing tissue culture fluid were produced; this material was pelleted,resuspended, and issued to various collaborating laboratories at approximately 160X of its original concentration. Production lots have been sent to investigators in both the U.S. and the U.S.S.R.

The production of Rauscher leukemia virus from BALB/c mouse plasma continued uninterrupted during the past year, with a total production in excess of 11,500 ml. of a 1:1 plasma:citrate preparation. Recipients of this material have included Drs. Spiegelman, Rich, Scolnick, Todaro, and Frankel, among others.

The contractor terminated production of Moloney sarcoma virus (MSV) at the end of November, 1974, having satisfied current Program requirements for this agent. The total volume of partially purified, gram-equivalent virus preparations from tumor extracts produced during this period was over 4,000 ml.

Production of feline leukemia virus (Rickard-subgroup A) in tissue culture roller bottles replaced production of MSV and was initiated early in

January, 1975. From the initiation of production to June, 1975, the contractor produced approximately 3,000 ml. of virus which had been concentrated 10X. Recipients of this virus included Drs. Todaro, Scolnick, and others.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera produced by this contractor has been extensively used by VCP researchers and is essential to the continuation of many important research projects presently being carried out in the Program.

Proposed Course: Production of needed strains of oncogenic viruses and their antisera will continue in volumes necessary to meet VCP needs.

Date Contract Initiated: June 4, 1962

Current Annual Level: $400,521

7. PROGRAM MANAGEMENT SEGMENT

Dr. J.B. Moloney, ADVO, DCCP, NCI, Chairman
Dr. Louis R. Sibal, OADVO, DCCP, NCI Executive Secretary

UNIVERSITY OF NORTH DAKOTA (NO1-CP6-0008)

Title: Quantitative Studies on the Transmission of Selected Herpesviruses and Type C RNA Viruses by Arthropods.

Contractor's Project Director: Dr. Robert G. Fischer

Project Officer (NCI): Dr. George J. Burton

Objectives: The major objective is to determine whether diseases induced by DNA or RNA viruses can be transmitted from infected animals to healthy susceptible animals by arthropods.

Major Findings: Insect transmission experiments will be completed with Marek's disease virus, murine cytomegalovirus (Henson strain) and Friend leukemia virus, using mosquitoes, flies, fleas, and other arthropods.

Significance to Biomedical Research and the Program of the Institute: Viruses may be transmitted biologically or mechanically by arthropod vectors. It is important to investigate whether oncogenic viruses present in infected animals are potentially transferable to man.

Proposed Course: Termination.

Date Contract Initiated: October 27, 1965

Current Annual Level: Extended without additional funds. This contract will be terminated December 31, 1975.

LITTON BIONETICS, INC. (NO1-CO-25423), BETHESDA, MARYLAND

Title: Operation and Maintenance of the Frederick Cancer Research Center at Frederick, Maryland

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Objectives:

A. To develop and establish large-scale virus production and purification capabilities on a continuing basis and to prepare special viral diagnostic and test reagents, to meet NCI program requirements.

B. To conduct developmental research relative to virus production of those oncogenic or suspected oncogenic viruses for which no established protocols exist or for which existing protocols have failed to consistently provide a suitable product, to develop and/or adapt techniques for accurate, reproducible assays of viral products.

C. To provide a capability for the evaluation of all products used or distributed within the Viral Oncology Program at the Frederick Cancer Research Center (FCRC) and elsewhere; to characterize the various viruses, viral components, and cell cultures and insure that those products meet or exceed NCI-VO Program quantitative and qualitative requirements.

D. To perform basic research in support of existing NCI programs pertaining to the role of virus in the etiology of human neoplasms; pursue research objectives, determined by NCI staff, reflecting those areas of scientific investigation that require special emphasis and/or expanded activities that can maximally utilize FCRC facilities.

E. To provide facilities and personnel for the applied collaborative biological safety research program of the NCI Office of Biohazards and Environmental Control; to develop safe laboratory practices and hazard control devices and methods to protect the laboratory worker, his experiments, and to insure the safety of the surrounding community.

F. To develop and implement a safety and environmental control program for the Frederick Cancer Research Center and to support applied and basic studies that will be performed by the Office of Biohazards and Environmental Control.

G. To operate an animal farm for the breeding of rodents to meet the needs of research programs at FCRC and for shipment to other NCI operations as production permits.

Major Findings:

Those projects that are completely funded by Viral Oncology are the following:

Project 1, Virus and Viral Reagent Production. During the period 2 February 1974 to 19 January 1975, 11,695 L of viruses were produced and 11,283 L were concentrated, yielding a total of 16,511 milliliters of concentrated and purified virus. Of this amount, 12,521 milliliters were released to Program Investigators, thereby fulfilling 100 percent of that amount requested by the Office of Program Resources and Logistics for investigators. Seventy-three percent of the virus produced was Rauscher murine leukemia virus (RLV). Except for special 70S RNA preparations of RLV, the final products were generally characterized as containing 5×10^{11} virus particles/ml, 3000 pico moles RDDP by endogenous assays and 300,000 pico moles RDDP by exogenous assay, and 0.5 to 5.0 mg protein/ml. Woolly monkey virus (SSV-1) production accounts for 25 percent of the virus produced. Final products of this virus yielded 10^{10} particles/ml, RDDP activity of about 50 pico moles/ml/hr, and protein content of about 0.5 mg/ml. Two other viruses (SA4B and E25t) were produced in relatively small amounts.

A system for the large-scale production and purification of a Rauscher murine leukemia virus product containing intact 70S RNA was developed. The system involves two sequential two-hour harvests of 96-hour production cultures, with complete processing, including concentration and purification of the culture fluids occurring in a single day. The products are positive for 70S RNA by the simultaneous detection assay. Investigators evaluating the product have reported that the material is unnicked and denatures to about 60 percent 34S RNA.

Laboratory-scale studies on the production and purification of Gross leukemia virus and gibbon ape lymphoma virus were initiated.

A computer program, encompassing all aspects of large-scale virus production, virus purification, product characterization, and release of virus concentrates from the FCRC Virus Repository, was designed and tested. The program contains three master files on: production, purification and product characterization, and inventory control. From these files information can be selected for purposes of production, purification and product analysis and interpretation, for distribution records, for availability of specific virus preparations within the FCRC Virus Repository and for report preparation.

Two viral proteins (p30 and RDDP) have been purified from RLV by detergent lysis, followed by sequential ion-exchange column chromatography. They appear homogeneous by SDS-acrylamide gel electrophoresis. Their respective monospecific antisera have been prepared in rabbits and goats. These four viral diagnostic reagents are distributed to investigators at the direction of the Office of Program Resources and Logistics, NCI. A single two hundred milliliter lot of concentrated RLV is processed each month. Since July 1974, a total of 4.4 mg of RDDP, 9.2 mg of p30, 48.25 mg of anti-RDDP IgG, 1020 ml of rabbit p30 antiserum, and 1880 ml of goat p30 antiserum have been produced. A total of 4.865 mg of RDDP, 41.52 mg of p30, 16.50 mg anti-RDDP IgG, and 127 ml of goat p30 antiserum have been distributed to Program investigators.

A total of 1.49 mg of RDDP, 15.16 mg of p30, 36.5 mg of anti-RDDP IgG and 1015 ml of rabbit and 1218 ml of goat p30 antiserum currently exist in the FCRC Virus Repository.

Project 2, Developmental Research. Studies were directed at defining culture conditions for increased production of infectious EBV (I-EBV). Methods developed for concentration, purification and storage included continuous-flow-pelletization and continuous flow-with-banding on sucrose on tartrate gradients. These procedures were implemented as protocols and demonstrated I-EBV production at levels of 36 liters per lot with infectivity titers averaging 5.0 log 10/ml of 1000 x virus concentrate. Additional studies were directed at improving assay methods for I-EBV including optimization of test conditions and Raji cell growth and application of the above techniques to production assay and characterization of B-95-8 derived transforming EBV with regard to virus titers, specific EBV antigens, and virus morphology. An in vitro system for production of MMTV was developed based on dexamethasone stimulation of Mm5mT/c$_1$ mouse mammary tumor cells. This system was implemented into large scale production with concomitant immunological (structural antigens) biochemical (viral reverse transcriptase) and biological (tumor induction) characterizations of the in vitro produced virus relative to mouse derived MMTV. Biochemical and immunological procedures involving cation specificity and radioimmunoassay, respectively, were developed for detection and quantitation of MMTV and murine leukemia virus expression. The electron microscopy laboratory supported the EBV and MMTV programs in terms of virus particle counts by negative stain, critical point assay, and thin section examination of virus and cell pellets. Additional efforts resulted in development of improved techniques for virus particle resolution, including fixatives and buffers, and implementation of techniques for virus particle quantitation including the Sharp technique. Methods were developed for purification of RDDP, p30, gp69/71, and RNase H from RLV. These techniques were applied to purification of these components from SSV-1. Monospecific antiserum was prepared against all of the purified components. Immunofluorescence and radioimmunoassay procedures are being developed and applied to the study of type C viruses.

Dr. O'Connor's SA4B Project. In studies with a candidate human virus, SA4B, cultures derived from a human liposarcoma were verified as being of human origin and not containing mycoplasma. They were shown to be carrying a virus which was released by induction techniques (IUDR and Dexamethasone). The parent cell was a low producer of this virus (STV); it was observed in thin sections of concentrated virus pellets but budding from cell surfaces was not detected. Uninduced cells contained an RDDP-like enzyme which was not neutralized by anti-murine p30 IgG. The enzymatic activity was neutralized to 25 percent by anti-SSV-1 RDDP IgG. The viral enzyme was not characterized due to insufficient quantities of STV. Also, early hybridization results with STV preparations indicated that this agent was not related to mouse, but only to non-human primate SSV-1 virus. The products used in the hybridization procedures were characterized by equilibrium sedimentation ultracentrifugation analysis using cesium sulfate gradients. However, radioimmunoassays with 5000-fold concentrated STV indicated a strong cross to both murine p30 and p15 antigens. On the basis of these results, this candidate human oncornavirus program was terminated.

Project 3, Product Characterization and Standardization. The Quality Control Unit has implemented procedures for carrying out exogenous and endogenous RDDP determination, simultaneous detection assays, total protein assays, complement fixation tests for RLV antigen, virus particle counts by negative staining and ultra-thin section with micrographic documentation, sterility testing for bacteriological, fungal and mycoplasmal entities, as well as in vivo and in vitro testing of RLV. Assay procedures have recently been implemented for determination of RDDP activity in MMTV preparation and for the titration of the early antigen of EBV.

A total of 3738 samples were tested during the January 1974-January 1975 interval. This level of effort, which was primarily in support of Project 1 program, represents a two-fold increase in the number of samples tested during the previous twelve-month period.

Project 13A, Herpesvirus Program for Support of Dr. Albert B. Sabin. The previously reported data on the existence of HSV-1 and HSV-2 nonvirion antigen and on the presence of specific CF antibodies to these neoantigens only in the sera of hyperimmunized guinea pigs or patients with certain types of cancer, could not be confirmed. The assumption that a mixture of two anticomplementary test components bound at least as much complement as the more anticomplementary ingredient was found to be incorrect for the reagents used in this study. The reverse was found to be true. Previous failure to observe some of the strict requirements of the complement fixation test could account for some of the previously unconfirmed data. As a result of the lack of specific confirmatory data, however, this work was terminated.

Project 13A.1, Virus Disease Modification. This project was operational from February 1974 through June 1974. The effect of tilorones, levamisole and other immunostimulating drugs on ameliorating the course of disease induced in mice by RLV and LSTRA leukemia was studied. Tissue culture studies directed towards understanding the in vitro activity of tilorones, levamisole, staphylococcal membranes, and BCNU were continued. The effect of these various immunostimulatory compounds on the activity of the viral reverse transcriptase was investigated. From June 1974 through November 1974 the Biochemistry effort was continued resulting in further studies on the mechanism of action of anthracycline compounds. The in vivo area of study continued on the immunosuppression caused by BCNU administration until November, at which time all work pertaining to this project ceased at FCRC.

Project 13A.2, Isolation and Characterization of Virus-Augmented Tumor Transplantation Antigens. A delayed hypersensitivity footpad assay has been developed to test for soluble tumor specific transplantation antigen (TSTA) induced by SV40. Hypertonic salt (3M KCl) solubilized TSTA gave a positive footpad reaction but did not protect against tumor cell challenge. Collaborative studies with Project 13A.4 have resulted in establishing a mouse model system for HSV.2 transformation. The time sequence of antigen and antibody formation and cell mediated immunity were examined in this murine system and salt soluble extracts from tumor cells are being prepared and tested for TSTA activity. Methodology has been developed for explanting mouse

mammary tumor virus-induced adenocarcinomas into tissue culture. TSTA experiments in this system are in progress. A new method for the analysis of aminosugars by gas chromatography has been developed.

Project 13A.3, Primate Virus Section. Research focused on the role of human (Epstein-Barr) and non-human (Herpesvirus saimiri and Herpesvirus ateles) primate oncogenic viruses in neoplasia. Immunological studies have explored the association of Epstein-Barr virus with American Burkitt's lymphoma and nasopharyngeal carcinoma and the seroepidemiology of Hodgkin's disease. The purification and characterization of complement fixing, membrane and nuclear antigens from EBV and HVS cell systems, in addition to analysis of HVS structural proteins, has been initiated. A new DNA polymerase has been detected in EBV-producer cell lines. Studies in monkeys have emphasized the persistence of HVS and HVS-specific antibodies in experimental animals which do not develop malignant lymphoma and/or lymphocytic leukemia. The effect of heteropolymeric anion on enhancing DNA virus titers was investigated.

Project 13A.4, Transformation with DNA Tumor Viruses and Chemical Carcinogens. A murine cell line (238) was transformed following infection of the cells with UV-inactivated herpes simplex virus type 2. The transformed parent cell line (H238) produces tumors with a 100 percent incidence in fully immunocompetent BALB/c mice inoculated with 10^6 cells. Indirect immunofluorescence tests revealed the presence of HSV specific antigens in the transformed cells. HSV.2 specific antibodies, capable of neutralizing HSV.2 but not HSV.1 virus infections, were found in sera from mice bearing tumors induced by the transformed cells. The transformed cells do not harbor infectious HSV.2 particles, nor could infectious HSV DNA be rescued. In the same model system the enhancement of transformation by herpes simplex virus (HSV) with certain chemical carcinogens was studied. Polycyclic aromatic hydrocarbons, e.g. DMBA, were used to pretreat primary BALB/c embryo (BE) cells and normal human fibroblast (HF) cells prior to infection with HSV. Cultures were then monitored for transformation. General characteristics of this system may provide insight and reagents for evaluating the relationship of HSV.2 to certain forms of human cancer.

Project 13A.5, Influence of Host Physiology on Oncogenesis and Cellular Control. Laboratory renovations having recently been completed, endogenous xenotropic viruses are now being studied for their physiological role in the host, emphasizing the interrelationship of the host-graft response and steroid-gene interaction on control of expression of the viral genome. The latter model system will consist of a Chinese hamster ovary cell system in which a type C virus is expressed as a result of treatment with cAMP. The mechanism by which viral expression is kept under control normally, and the control is "broken" in the presence of cAMP, will be investigated. Details of the host-graft portion of this program are not available at this time. Identification of the virus and isolation of a cAMP binding protein will be undertaken.

Project 13A.6, Developmental Electron Microscopy. Innovative utilization of the most recent electron optics instrumentation and techniques by the Developmental E.M. Lab during the past year have resulted in several important contributions to the Viral Oncology research program. A precise and reliable virus quantitation technique has been developed (JNCI 53:1099-1102, 1974) which eliminates the problem of operator fatigue. A definitive study of morphological characteristics of human tumor cell lines has been submitted for publication and will provide the basis for study of these cell lines in their interactions with chemicals and viruses. A collaborative effort has elucidated the effects of certain chemical compounds on virus production. A research program employing energy-dispersive X-ray analysis and immunochemical methods has been initiated.

Project 13A.7, Role of Endogenous Mouse Type C RNA Viruses in Naturally-Occurring Disease. A total of 1918 mice are being held under controlled environmental conditions. Spontaneous development of murine leukemia is being monitored by assessment of gross pathological lesions. Experiments are being initiated to assess genetic and chemical factors related to the induction of endogenous type C RNA viruses and their role in the pathogenesis of murine leukemia.

Project 13B, National and International Cancer Laboratory Liaison. Laboratory facilities for long and short-term visiting foreign scientists have been established. A highly successful international workshop on Epstein-Barr virus production, concentration and purification was planned and held at FCRC in February 1975. Other activities included the writing of a laboratory manual on immunological methods useful in cancer research, and participating in the efforts of the International Agency for Research on Cancer and WHO/FAO programs to coordinate various efforts in oncogenic herpesvirus research.

Project 5, Biohazards and Environmental Control. The chemical disinfection of Rauscher leukemia virus with alcohols, iodophors and quaternary ammonium compounds has continued. Concentrations of 35% ethanol and 0.5% Wescodyne, are capable of inactivating 6.3×10^7 infectious units of purified virus in 30 and 10 seconds respectively; no detectable infectious virus was found post-treatment. The evaluation of a mass airflow animal containment system, using Newcastle disease virus in chickens and Sendai virus in mice, was completed. At airflows of 100 and 50 linear feet per minute, containment of aerosol spread of the virus was achieved. There was no indication of cross-infection in the containment facility. While environmental conditions play an important role in the rate of contamination with an open bench or Class I cabinet, effective use of Class II laminar flow biological safety cabinets prevented any overt contamination. Other sources of contamination were investigated and found to be only point sources of contamination. In other tests, there was little evidence of residual formaldehyde found in a decontaminated Class II cabinet after 24 hours of airwash. Eighteen cabinets have been received and evaluated for compliance to the NIH safety specifications. A variety of tests were performed to study the design and operation of Class II cabinets, including the use of fluorescein to determine filter integrity, the maximum biological challenge, lateral spread of a generated

aerosol, personnel and product protection tests, and hazard assessment of biomedical equipment. Development of the annual safety program for this year is proceeding. The symposium for this year will be "Biohazards and Zoonotic Problems of Primate Procurement, Quarantine, and Research." Development of information data sheets has been initiated for incorporation into the "NCI Safety Standards for Research Involving Oncogenic Viruses."

Those projects that are partially funded by Viral Oncology are the following:

Project 4, Environmental Control. There were six lost work-time injuries resulting in a total of 25 lost workdays in 1974. In 1973, there were four lost work-time injuries resulting in a total of 15 lost workdays. However, the frequency rate which is based on the workdays lost in relationship to the employee population was 5.04 in 1974 and 5.27 in 1973. Special emphasis and effort during the year were directed towards safety in the use, handling, storage and disposal of chemical carcinogens. Personnel from Project 4 served on the DHEW Safety Committee that developed the DHEW Safety Standards for Laboratory Operations Involving Chemical Carcinogens. These safety standards were used as a basis for developing the FCRC Standards for Safety in Research Involving Chemical Carcinogens. The various control, inspection and training programs, as well as the short-term, applied research projects in the areas of biological, radiological and industrial safety were carried out without significant difficulties. The Medical Services Department continues to provide excellent medical service for FCRC employees. VO supports 35% of this work.

Project 12, Animal Breeding. During calendar year 1974, the Animal Farm produced 433,007 laboratory animals of 26 different strains, or species, for use in cancer research programs. The idea of publishing a chart listing the availability of animals resulted in the effective utilization of most animals produced and reduced sacrificing of unneeded costly research animals to an acceptable minimum. The Animal Farm is rapidly approaching full utilization of the available facilities at the FCRC. Analyses are planned to evaluate the best proposed utilization of the facilities. The Strain 2/N guinea pig colony was expanded by 20 percent with further expansion contingent upon elimination of the Hartley guinea pig colony. Expansion of the Strain 13/N guinea pig colony is still in progress. Also of interest was the phasing out of the NAW/FD rabbit colony when it became apparent that the numbers required were too small to justify keeping the colony. VO supports 25% of this work.

Significance to Biomedical Research and the Program of the Institute:

FCRC Projects in the Viral Oncology program have continued to play an increasingly significant role in providing high quality biological materials that are a necessary requirement for NCI programs in virology, immunology, chemotherapy, molecular biology, genetics and electron microscopy. The work will also continue to provide specific information to FCRC and outside laboratories on handling of infectious biological materials as related to biohazards and environmental control problems. In addition, the basic research effort augmented the Cancer Program by providing information on problems related to the elucidation of human neoplastic disease relative to detection,

prevention, and control. Finally plans also included the availability of high caliber laboratory facilities for special investigations, visiting scientists, and an advanced workshop at FCRC involving recognized experts from both this country and abroad.

Proposed Course:

To take increasing advantage of project accomplishment in previous years and to reflect the need to further implement research in support of Viral Oncology and the NCI's program of coordinated research on viruses as etiologic agents of cancer, program effort for the fourth year of operations will be restructured to create the following laboratories: (1) DNA Virus Laboratory, (2) RNA Virus Laboratory, (3) Disease Control Laboratory, and (4) Virus/Reagent Production and Developmental Research Laboratory. It will be the function of these Laboratories to conduct basic or exploratory research on programs that are or could be vital to the Cancer Program. The research in the Laboratories will include some activities previously performed under Projects 1, 2, 3 and 13, and should take full advantage of unique opportunities at the FCRC to perform investigations for which a program need exists. In addition, the contractor will provide necessary facilities and support services for viral oncology investigators whose NCI operations are located at FCRC. Also as part of the FCRC Viral Oncology Program, there will be included the effort on applied research for Biohazards and Environmental Control, previously designated as Project 5.

It should be reemphasized that the FCRC Viral Oncology effort is to be performed as a total integrated activity that will allow for a high degree of flexibility in response to overall program needs and for broad latitude in creating and pursuing fundamental research objectives both in-house and on a collaborative basis with outside acknowledged experts. As work in the given areas is performed, particular research objectives may receive changes in emphasis; objectives may be modified and/or previously established ones postponed or eliminated.

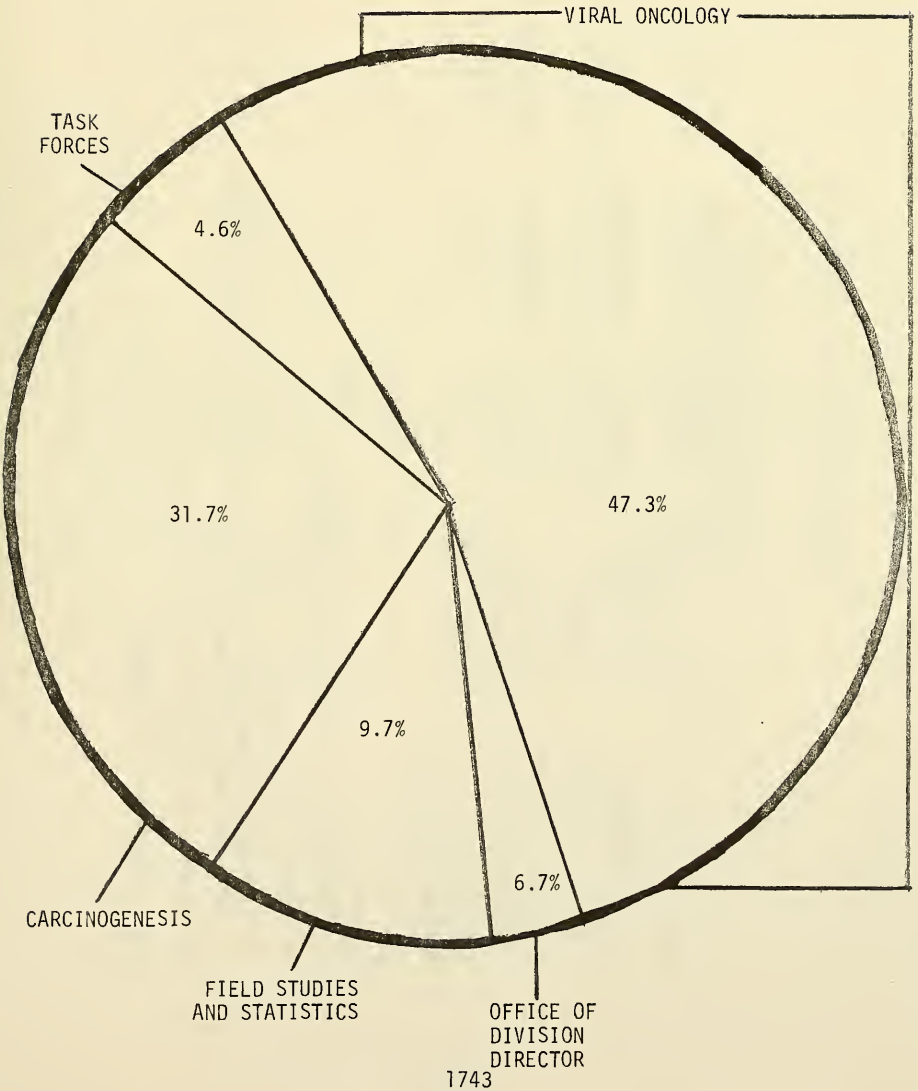
Date Contract Initiated: June 26, 1972

Current Annual Level: $4,801,722 (This includes only Viral Oncology's portion of Contract NO1-CO-25423)

NATIONAL CANCER INSTITUTE

Division of Cancer Cause and Prevention

Current Distribution of Funds - Fiscal Year 1975



DIVISION OF CANCER CAUSE AND PREVENTION

NCI

Table of Ratios of Contracts to
Intramural Effort Based on
Estimated Current Level of Expenditure**

JUNE 30, 1975

	ODD	FIELD STUDIES AND STATISTICS	CARCINOGENESIS	VIRAL ONCOLOGY	TASK FORCES	TOTAL
Total	8,171,000	11,924,000	38,794,000	57,884,000	5,580,000	122,353,000
Contracts	6,779,000	8,809,000	34,181,000	49,885,000	5,125,000 1/	104,779,000
Intramural*	1,392,000	3,115,000	4,613,000	7,999,000	455,000 2/	17,574,000
Ratios: Contract to Intramural	0.5:1	2.8:1	7.4:1	6.2:1	11.3:1	6.0:1

1744

---

* Includes cost of managing contract activities

** Based on FY 1975 President's Budget

1/ Includes $3,675,000 for Lung Cancer (Carcinogenesis Area); and $1,450,000 for Breast Cancer (Viral Oncology Area).

2/ Direct operations for Lung Cancer (Carcinogenesis Area)

Note: Construction not included.

DCCP - FINANCIAL PLAN

FY 1975 - 691.M Level

(000)

Area	P.S.	O.O.	Total I.H.	Contracts	TOTAL
Off. of Div. Dir.	$ 995	$ 397	$ 1,392	$ 6,779	$ 8,171
Viral Oncology	4,702	3,297	7,999	49,885	57,884
Carcinogenesis	2,810	1,803	4,613	34,181	38,794
Field Studies & Statistics	2,080	1,035	3,115	8,809	11,924
Total	$10,587	$6,532	$17,119	$99,654	$116,773
Breast Cancer				$ 1,450	$ 1,450
Lung Cancer	$ 273	$ 182	$ 455	$ 3,675	$ 4,130
	$10,860	$6,714	$17,574	$104,779	$122,353

OFFICE OF DIVISION DIRECTOR

Peters, James A.	Director
Gori, Gio B.	Deputy Director
Flamm, W. Gary	Assistant Director
Kraybill, Herman F.	Sci. Coord. for Environ. Cancer
Depue, Robert H., Jr.	Scientist Director
Owen, Thomas B.	Microbiologist

Frederick Cancer Research Center

Payne, William W.	Sci. Coordinator for FCRC
Carey, James K.	Expert
Bourland, Orley R., Jr.	Biochemical Engineer

Administrative Management Branch

Miller, John M.	Administrative Officer, Chief
Olimpio, Nicholas A.	Program Analyst
Velthuis, Robert, Jr.	Administrative Officer, Viral Oncology
Crane, Damian	Administrative Officer, Carcinogenesis
Doyle, James D.	Admin. Officer, Field Studies & Stat.

Office of Associate Director for Field Studies & Statistics

Schneiderman, Marvin A.	Associate Director
Fox, Bernard H.	Research Psychologist
Goldsmith, John R.	Environ. Cancer Epidemiologist (IPA)

Biometry Branch

Office of the Chief

Haenszel, William M.	Chief
Cutler, Sidney J.	Associate Chief
Murray, James L.	Vet. Officer Director
Levin, David L.	Sr. Surgeon
Brown, Charles C.	Res. Math. Statistician
Locke, Frances B.	Statistician

Automatic Data Processing Management Section

Weiss, Theodore	Digital Comp. Sys. Admin., Head
Larson, James E.	Computer Systems Analyst
Oslik, Norman	Computer Systems Analyst
Stump, James M.	Computer Specialist
Cusano, Mary M.	Computer Specialist
Van Holten, Valerie	Computer Specialist
Gales, Altamease A.	Computer Programmer

Clinical & Diagnostic Trials Section

Byar, David P.	Medical Officer, Head
Gail, Mitchell H.	Sr. Surgeon
Green, Sylvan B.	Surgeon
Corle, Donald K.	Computer Systems Analyst
Koziol, James A.	Staff Fellow
Muenz, Larry R.	Staff Fellow (France)

Demography Section

Young, John L., Jr.	Sr. Scientist
King, Haitung	Research Sociologist
Scotto, Joseph	Sr. Health Services Officer
Connelly, Roger R.	Statistician (Health)
Percy, Constance L.	Statistician (Health)
Horm, John W.	Sr. Asst. Health Serv. Officer
Devesa, Susan S.	Statistician

End Results Section

Myers, Max H.	Res. Mathematician, Head
Lourie, William I., Jr.	Statistician (Health)
Axtell, Lillian M.	Statistician (Health)
Hankey, Benjamin F.	Res. Math. Statistician
Prorok, Philip C.	Staff Fellow
Baranovsky, Anne	Statistician (Health)
Heise, Herman W.	Statistician (Health)
Shambaugh, Evelyn M.	Statistician (Health)
Asire, Ardyce J.	Statistician (Health)

Field Liaison Section

Geller, Harvey	Statistician (Health), Head
Baylis, Paula H.	Supv. Stat. Officer

Mathematical Statistics & Applied Mathematics Section

Gart, John J.	Res. Mathematician, Head
Connor, Robert J.	Res. Math. Statistician
Pettigrew, Hugh M.	Res. Mathematician
Thomas, Donald G.	Res. Math. Statistician
Layard, M. W.	Sr. Staff Fellow
Nam, Jun-Mo	Math. Statistician
Tarone, Robert E.	Staff Fellow
Smith, Alroy M.	Statistician (Health)

Epidemiology Branch

Office of the Chief

Miller, Robert W.	Chief
Fraumeni, Joseph F., Jr.	Associate Chief
Blattner, William A.	Surgeon
Blot, William J.	Statistician (Health)

Clinical Genetics Section

Mulvihill, John J.	Surgeon, Head
Everson, Richard B.	Surgeon
Pendergrass, Thomas W.	Surgeon

Environmental Studies Section

Hoover, Robert N.	Surgeon, Head
Mason, Thomas J.	Sr. Staff Fellow
McKay, Frank W., Jr.	Computer Systems Analyst

Epizoology Section

Priester, William A., Jr.	Vet. Officer Dir., Head
Hayes, Howard M.	Vet. Med. Officer

Special Cancer Studies Section

Miller, Robert W.	Acting Head

Boston Field Station

Li, Frederick P.	Sr. Surgeon, Head

Office of Associate Director for Carcinogenesis

Saffiotti, Umberto	Associate Director
Cooper, John A., II	Chemist, Deputy Assoc. Director
Bates, Richard R.	Sci. Coord. for Carc., FCRC
Warner, Charles R.	Chemist
Chen, Beatrice	Staff Fellow
Idoine, Jane B.	Res. Chemist (FCRC)

Office of Coordinator for Collaborative Research

Heim, Allen H.	Microbiologist, Head
Pledger, Richard A.	Microbiologist
Dunkel, Virginia C.	Microbiologist
Kaplan, Ann E.	Chemist
Litwack, Marcia D.	Chemist
Smith, Carl E.	Chemist
Sontag, James M.	Microbiologist (Sci. Adm.)

Biology Branch

Office of the Chief

Rapp, Herbert J.	Chief
Borsos, Tibor	Associate Chief
Bast, Robert C., Jr.	Surgeon
Okuda, Takao	Visiting Fellow

Cellular Immunity Section

Zbar, Berton	Sr. Surgeon, Head
Harmel, Richard B.	Surgeon

Cytogenetics & Cytology Section

DiPaolo, Joseph A.	Res. Pharmacologist, Head
Evans, Charles H., Jr.	Sr. Surgeon
Myhr, Brian C.	Sr. Staff Fellow
Popescu, Nicholae C.	Visiting Associate
Donovan, Paul J.	Chemist
Nelson, Richard	Biologist

Immunochemistry Section

Borsos, Tibor	Res. Chemist, Head
Ohanian, S. H.	Res. Microbiologist
Hooks, Barbara D.	Staff Fellow
Boyle, Michael D. P.	Visiting Fellow

Tumor Antigen Section

Leonard, Edward J.	Medical Director, Head
Meltzer, Monte S.	Surgeon
Smith, Howard G.	Surgeon
Lett-Brown, Michael	Visiting Fellow

Chemistry Branch

Office of the Chief

Gelboin, Harry V.	Chief
Selkirk, James K.	Sr. Staff Fellow
Wiebel, Friedrick J.	Sr. Staff Fellow
Nemoto, Nobuo	Visiting Fellow

Protein Section

Peacock, Andrew C.	Res. Chemist, Head
Green, Marie R.	Res. Chemist
Bunting, Sylvia L.	Chemist
Pastewka, Jullia V.	Chemist
Nishinaga, Keiji	Visiting Fellow

Chemistry Branch (continued)

Nucleic Acids Section

Dingman, C. Wesley, II	Medical Officer, Head
Kakefunda, Tsuyoshi	Medical Officer (Res.)
Day, Rufus S., III	Sr. Staff Fellow
Kakumaga, Takeo	Visiting Scientist

Cell Growth Regulation Section

Bader, John P.	Res. Microbiologist, Head
Hatfield, Dolph	Res. Biologist
Harris, Rudolph	Staff Fellow
Okazaki, Takumi	Visiting Fellow

Molecular Carcinogenesis Section

Gelboin, Harry V.	Acting Head

Experimental Pathology Branch

Office of the Chief

Saffiotti, Umberto	Chief
Squire, Robert A.	Associate Chief
Yamamoto, Richard S.	Res. Chemist

In Vitro Pathogenesis Section

Yuspa, Stuart H.	Sr. Surgeon, Head
Hennings, Henry	Sr. Staff Fellow
Lichti, Ulrike F.	Res. Chemist
Morgan, David L.	Biologist

Perinatal Carcinogenesis Section

Rice, Jerry M.	Sr. Scientist, Head
Joshi, Sewa R.	Sr. Staff Fellow

Tumor Pathology Section

Squire, Robert A.	Expert, Head
Lingeman, Carolyn H.	Med. Officer (Path.)
Barrett, Margaret D.	Biologist
Levitt, Morton, N.	Surgeon

Lung Cancer Branch

Office of the Chief

Sporn, Michael B.	Medical Director, Chief
Reese, David H.	Staff Fellow
Smith, Joseph M.	Biologist
Van Haaften, C.	Visiting Fellow
Frot-Coutaz, Jacques	Visiting Fellow

Differentiation Control Section

DeLuca, Luigi	Chemist, Head

Lung Cancer Pathogenesis Section

Sporn, Michael B.	Acting Head
Harris, Curtis C.	Surgeon
Kaufman, David G.	Surgeon
Frank, Arthur L.	Surgeon

Carcinogen Bioassay and Program Resources Branch

Office of the Chief

Page, Norbert P.	Vet. Officer Dir., Chief
Cameron, Thomas P.	Vet. Officer Dir.,
Cueto, Cipriano	Pharmacologist
Siegel, Sidney	Biologist
Linhart, Mary S.	Computer Programmer

Carcinogen Metabolism and Toxicology Branch

Office of the Chief

Weisburger, Elizabeth K.	Scientist Dir., Chief
Silverman, Sidney J.	Res. Microbiologist (FCRC)
Evarts, Ritva P.	Visiting Scientist
Gothoskor, Sunanda	Visiting Fellow

Analytical Chemistry Unit

Keefer, Larry K.	Sr. Staff Fellow
Roller, Peter P.	Staff Fellow

Metabolic Studies Section

Weisburger, Elizabeth K.	Acting Head
Poirier, Lionel A.	Res. Chemist
Grantham, Preston H.	Res. Chemist

Office of Associate Director for Viral Oncology

Moloney, J. B.	Associate Director
Sibal, Louis R.	Deputy Assoc. Director
Hearn, Henry J.	Microbiologist (FCRC)
Zeve, Victor H.	Res. Biologist (FCRC)
Jordan, Elke	Chemist
Schlom, Jeffrey	Microbiologist
West, David L.	San. Engineer
Goldberg, Robert J.	Staff Fellow

Office of Biohazards & Environmental Control

Hellman, Alfred	Scientist Dir., Chief
Sun, Bill N.	Sr. Staff Fellow
Barbeito, Manuel S.	Microbiologist
Keefer, Garrett V.	Microbiologist

Biohazards Research Section

Fowler, Arnold K.	Sr. Scientist, Head
Steinman, Harry G.	Supv. Chemist
Strickland, James E.	Sr. Staff Fellow
Kouttab, Nicola M.	Visiting Fellow

Environmental Control Section

Hellman, Alfred	Acting Head

Office of the Coordinator of Ultrastructural Studies

Dalton, Albert J.	Res. Biologist, Coordinator
Chopra, Harish C.	Res. Microbiologist
Bader, Artrice V.	Res. Biologist

Virus Studies Section

Heine, Ursula I.	Res. Microbiologist, Head
Suskind, R. Gerald	Medical Director
Dahlberg, John E.	Sr. Staff Fellow
Weber, George H.	Sr. Staff Fellow
Elliott, Benjamin F., Jr.	Bio. Lab. Tech.

Office of Program Resources & Logistics

Gruber, Jack	Microbiologist, Chief
Holdenried, Robert	Scientist Director
Howell, David M.	Microbiologist
Sekely, Lea I.	Microbiologist
Varrato, Wilma	Computer Programmer

Viral Biology Branch

Office of the Chief

Manaker, Robert A.	Chief
Chirigos, Michael A.	Associate Chief
Reisinger, Robert C.	Veterinarian
Guss, Maurice L.	Microbiologist
Stansly, Pauline	Biologist

Cell Biology Section

Boone, Charles W.	Medical Officer, Head
Davis, Stephen	Surgeon
Orme, Thomas W.	Sr. Staff Fellow
Takeichi, Noritoshi	Visiting Scientist
O'Brien, Stephen J.	Staff Fellow
Paranjpe, Meera S.	Staff Fellow
Koegel, Robert J.	Res. Chemist

Experimental Pathology Section

Manaker, Robert A.	Acting Head

Human Tumor Studies Section

Vande Woude, George F.	Res. Chemist, Head
Ascione, Richard	Res. Chemist
Ebert, Paul S.	Res. Chemist
Andrese, Angelo P.	Staff Fellow
Graham, Bettie J.	Staff Fellow
Robey, William G.	Staff Fellow
Simonds, Josephine A.	Staff Fellow

Microbiology Section

Pearson, Gary R.	Microbiologist, Head
Gravell, Maneth	Res. Microbiologist
Redmon, Lena W.	Biologist

Virus & Disease Modification Section

Chirigos, Michael A.	Res. Chemist, Head
Woods, Wilna A.	Res. Microbiologist
Pearson, John W.	Microbiologist
Fliegelman, Martin J.	Surgeon
Mohr, Stephen J.	Surgeon
Papas, Takis	Sr. Staff Fellow
Pry, Thomas W.	Microbiologist

Viral Carcinogenesis Branch

Office of the Chief

Huebner, Robert J.	Medical Director, Chief
Duff, James T.	Associate Chief
Arnstein, Paul	Vet. Officer Dir. (California)
Kelloff, Gary J.	Sr. Stafff Fellow
Streicher, Harriet L.	Program Assistant
Capps, Worth I.	Bio. Lab. Tech.

Ecology and Epizoology Section

Sarma, Padman S.	Res. Microbiologist, Head
Estes, John D.	Bio. Lab. Tech. (California)

Molecular Biology Section

Aaronson, Stuart A.	Medical Officer, Head
Bensinger, William I.	SA Surgeon
Portugal, Franklin H.	Sr. Staff Fellow
Stephenson, J. R.	Sr. Staff Fellow
Tronick, Steven R.	Sr. Staff Fellow
Reynolds, Roberta K.	Microbiologist

Solid Tumor Virus Section

Hampar, Berge	Dent. Dir., Head
Brown, Ashley	Res. Scientist
Gurmit, Sing L. A.	Visiting Fellow

Viral Genetics Section

Parks, Wade P.	Sr. Surgeon, Head
Wolford, Ronald G.	Microbiologist

Serology Unit

Hill, Paul R.	Supv. Bio. Lab. Tech.

Trailer Unit (Poolesville)

Lane, William T.	Bio. Lab. Tech.

Viral Leukemia and Lymphoma Branch

Office of the Chief

Todaro, George J.	Medical Officer, Chief
Sherr, Charles J.	Surgeon
Leiseca, Sergio A.	Res. Biologist
Benveniste, Raoul E.	Sr. Staff Fellow
DeLarco, Joseph E.	Sr. Staff Fellow
Callahan, Robert	Staff Fellow
Duran - Troise, G.	Visiting Fellow
Guegan, Jean	Visiting Fellow
Sen, Arup	Visiting Fellow

Clinical Studies Section

Levine, Paul H.	Medical Officer, Head
Burton, George J.	Scientist Dir.
Gaylord, Clarice E.	Health Scientist Administrator
Perkins, Ida Virginia	Microbiologist (Ghana)
Hesse, Jette	Visiting Associate

Genetics Section

Scolnick, Edward M.	Medical Officer, Head
Silverman, Andrew Y.	Surgeon
Levin, Reuven	Visiting Fellow

Immunology Section

Aoki, Tadao	Medical Officer, Head
Plata, E. J.	Microbiologist
Brandchaft, Phyllis B.	Microbiologist
Martin, W. J.	Visiting Scientist
Okazaki, Etsuo	Visiting Scientist
Kvedar, John P.	Bio. Technologist
Kimura, Hideki	Visiting Fellow

Primate Virus Section (FCRC)

Ablashi, Dharam V.	Res. Microbiologist, Head
Easton, John M.	Sr. Surgeon
Luetzeler, Josef	Visiting Associate
Armstrong, Gary R.	Biologist

Tumor Virus Section

Fischinger, Peter J.	Medical Officer, Head
Frankel, Arthur E.	SA Surgeon
Peebles, Paul T.	Sr. San. Engineer
Haapala, Daniel K.	Microbiologist
Phillips, Leo A.	Microbiologist
Nomura, Shigeko	Visiting Scientist
Pang, Roy H.	Visiting Fellow

Viral Biochemistry Section

Bassin, Robert H.	Microbiologist, Head
Gerwin, Brenda I.	Sr. Staff Fellow
Canivet, Martine	Visiting Associate

Viral Pathology Section

Gazdar, Adi F.	Medical Officer, Head
Merwin, Ruth M.	Research Biologist
Oie, Herbert K.	Staff Fellow
Park, Sang Shin	Visiting Fellow
Russell, Edward K.	Chemist
Sims, Harvie L.	Bio. Lab. Tech.

7435 3

DATE DUE

GAYLORD

PRINTED IN U.S.A.

LIBRARY
NIH
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY
4 0121 9637

WERT
BOOKBINDING
MIDDLETOWN, PA
NOVEMBER 75
We're Quality Bound

NIH LIBRARY
3 1496 00185 1313